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Faunistic Note on a Collection of Helminthic Material from Palestine.

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In April, 1931, a collection of helminth parasites was received by the Imperial Bureau of Agricultural Parasitology from Mr. Bodkin, the Government Entomologist at Jerusalem. The specimens appear to have been collected by Native Meat Inspectors from slaughter houses, farms, etc., ranging through eleven different localities, from Hebron and Gaza, west of the Dead Sea, in the south ; to Acre and Safad, north of the Sea of Galilee. The collection comprised 117 bottles of material, much of which had been rather poorly preserved. For such a large assortment of material the number of species is relatively small, as so many of the parasites were sent in duplicate. There does not appear to be any species new to science, and the following note is intended as a contribution to the zoögeography of a country whose parasitic fauna is somewhat inadequately known.

The writer is greatly indebted to Professor R. T. Leiper, F.R.S., for placing the material at his disposal and to the Imperial Bureau of Agricultural Parasitology for much helpful data concerning the collection.

The collection was found to comprise in all : One species of Trematode, 12 spp., representing seven genera, of Cestodes and 12 spp., representing 10 genera, of Nematodes. The range of hosts examined is wide, including the following domestic and farm animals : Sheep, goat, oxen, buffalo, cattle, horse, rabbit, camel, pig and fowl.

CESTODES.

The cestode material includes 11 species, three of which are cystic stages of members of the genus *Tænia*.

Fam.: *TÆNIIDÆ* Ludwig, 1886.

Genus: Echinococcus Rudolphi, 1901.

Echinococcus granulosus (Batsch, 1786).

This collection contains 24 cases of hydatid disease collected from the following localities: Safad, Haifa, Jenin, Ramallah, Jerusalem and Bethlehem. One rather curious multilocular cyst is described as coming from the "bile" of a sheep—probably meaning the gall bladder. This cyst measures seven centimeters in length, is shaped like a sausage and has numerous spherical or subspherical diverticuli and is infertile. The remaining cysts examined are all of normal spherical form, though varying greatly in age. Some of the hepatic infections consist of numerous very young cysts. There are many infertile or calcified specimens. The specimens are taken from the following hosts: Sheep, goat, cattle and camel. The infected organs are in all cases either liver or lungs: the 24 cases including 10 hepatic and 15 pulmonary infections—one case showing a double infection.

Genus: Tænia Linn., 1758.

Cysticercus tenuicollis (= larva of *T. hydatigena* (Pallas, 1766)).

Twenty cases have to be recorded: 10 from sheep and 10 from goats. The position of this characteristic bladder worm in the host appears to vary considerably. Of the 20 cases under consideration two are from the intestines, three from peritoneal positions, two from the pancreas, two from the lung, two from the stomach and seven from the liver. Specimens were collected from the following localities: Safad, Acre, Haifa, Bethlehem, Ramallah and Jaffa. The great majority were collected at Safad, which would appear to be an enzootic focus for this worm as well as for hydatid.

Cysticercus bovis (= larva of *T. saginata* (Goeze, 1782)).

Five cases are included. All are from cattle. In three cases the cysts are in voluntary muscles, including those of the head and tongue, and in two cases they are in the cardiac muscles. The material was collected at five different localities: Jaffa, Ramallah, Jerusalem, Bethlehem and Haifa.

Cysticercus cellulosæ (= larva of *T. solium* (Linn., 1758)).

Four cases, all from pigs at Haifa. Two are from " muscles " (presumably voluntary), one from cardiac muscle and one from the lung—an unusual situation.

Genus : Multiceps Goeze, 1782.

Multiceps multiceps (Leske, 1780) Hall, 1910.

A single case of this common cœnurus is included. The specimen is from the brain of a goat. Locality : S. H. Safad.

Fam. : *ANOPLOCEPHALIDÆ* Fuhrm., 1907.
Sub-fam. : *ANOPLOCEPHALINÆ* Fuhrm., 1907.

Genus : Moniezia Blanchard, 1891.

Moniezia expansa (Rudolphi, 1810).

Two bottles were found to contain this worm. The specimens are from the intestines of a sheep from Safad in one case ; and in the other case from the small intestine of a goat from Khan Yunis. The diagnosis of the latter case is not entirely above doubt. The strobilæ are narrower than is normal for *M. expansa* and probably represent an unusually small strain. The interproglotidal glands are of the rosette pattern. In this it closely resembles the smaller *M. trigonophora* (Stiles and Hassall, 1893). There appears to be no definite morphological criterion by which these two species can be distinguished. (Baer, pp. 66-74.) Since *M. trigonophora* is not recorded from *Capra hircus* of which *M. expansa* is a well known parasite, the present material is identified as the latter.

Moniezia denticulata (Rudolphi, 1810).

Three cases. In the first the worms are taken from the intestines of a sheep and in the second from that of a kid. Both cases are from Safad. A single specimen is also included in a bottle of *Avitellina*—also from sheep at Safad. Diagnosis is based upon this species being the only Old World *Moniezia* in which the interproglotidal glands are absent. (Baer, p. 74.)

Moniezia benedeni (Moniez, 1879).

Only one case is included : from the intestines of a goat at Acre. The interproglotidal glands are evident, but the material is distinguished from

M. expansa by the larger eggs. (*M. benedeni* 60–80μ, *M. expansa* 50–60μ).

Genus: AVITELLINA Gough, 1911.

Avitellina centripunctata (Rivolta, 1874).

From the intestines of sheep and goats. Localities: Khan Yunis, Safad and Acre. There are three cases altogether.

Genus: HELICTOMETRA Baer, 1927.

Helictometra giardi (Moniez, 1879).

Three cases are included. In one case, from Gaza, the host is not given. The other two cases are respectively from sheep at Jenin and from cattle at Haifa. In all cases the position in the host is in the intestines.

The genus was erected by Baer in 1927 to comprise the worm *Tænia giardi* (Moniez, 1879) (= *Thysanosoma giardi* (Moniez) Stiles and Hassall, 1893). The present material agrees very closely with Baer's description and figure (p. 135, pl. III). There appears to be only one pair of excretory canals present in these specimens, whereas Baer describes two pairs, and the spines on the cirrus described by him are not apparent in this case. Also the antiporal group of testes appears somewhat more numerous than as stated by Baer. However, in all other details the individuals under examination show such close agreement with Baer's observations that it is not felt that a new species can be based upon these minor deviations from the type. The outstanding characters are the transverse undulating uterus, the tightly coiled vas deferens, small cirrus sac and bi-lateral arrangement of the testes in two lateral fields of unequal size.

Fam.: *DILEPIDIDÆ* Railliet and Henry, 1909.

Sub-fam.: *DILEPIDINÆ* Fuhrm., 1907.

Genus: CHOANOTÆNIA Railliet, 1896.

Choanotænia infundibuliformis (Goeze, 1782) Railliet, 1896.

This small tapeworm is represented in the collection by a number of specimens from the intestines of fowls.

TREMATODA.

The trematodes are represented in this collection only by *Fasciola hepatica* Linn., 1758. The material comprises 21 cases of liver fluke disease. The hosts include sheep, goat, cattle and domestic buffalo: only one case being recorded from each of the last two. The localities from which the cases were collected are: Jaffa, Bethlehem, Gaza, Acre, Nablus, Jenin, Ramallah, Hebron, Haifa, Safad and Tulkaren. This includes nearly all the collecting centres. The parasite is obviously widely and uniformly distributed throughout Palestine.

NEMATODA.

The nematode material proved to comprise 12 spp., representing 10 genera. The species included two Trichostrongyles, three Metastrongyles, one Ascarid, one Œsophagostome, three Heterakids and one Filariid. The hosts from which nematodes were taken are: Sheep, goat, cattle, horse and fowl.

Fam.: *METASTRONGYLIDÆ* Leiper, 1908.

Sub-fam.: *METASTRONGYLINÆ* Leiper, 1908.

Genus: DICTYOCAULUS Railliet and Henry, 1907.

This genus is represented in the collection by 12 (or 13) cases of *Dictyocaulus filaria* (Rudolphi, 1809) from sheep and goats, and one case of *D. viviparus* (Bloch, 1872) from cattle. Both species are responsible for verminous bronchitis or "husk" in their respective hosts. The single case of *D. viviparus* is from the bronchi of cattle from Acre. The remaining cases, all from the bronchi of sheep and goats, have been taken from the following localities: Ramallah, Bethlehem, Jaffa, Jenin, Hebron, Jerusalem, Gaza, Nablus and Acre.

Genus: PROTOSTRONGYLUS Kamensky, 1905.

(= SYNTHETOCAULUS Railliet and Henry, 1907).

The genus is represented by adults of *Protostrongylus rufescens* (Leuckart, 1865) from the lungs of a goat and also by larvæ of *Protostrongylus* sp. from the same source.

Fam.: *TRICHOSTRONGYLIDÆ* Leiper, 1912.
Sub-fam.: *TRICHOSTRONGYLINÆ* Leiper, 1908.

Genus: HÆMONCHUS Cobb, 1898.

Hæmonchus contortus (Rudolphi, 1803).

One case only; a dual infection of the 4th stomach (abomasum) of a goat with this worm and *Chabertia ovina*. The locality is Hebron.

Genus: OSTERTAGIA Ransom, 1907.

Ostertagia circumcincta (Stadelmann, 1894).

Here recorded from the 4th stomach (abomasum) of a goat. It occurs also in sheep and antelopes.

Fam.: *STRONGYLIDÆ* Baird, 1853.
Sub-fam.: *ŒSOPHAGOSTOMINÆ* Railliet, 1915.

Genus: CHABERTIA Railliet and Henry, 1909.

Chabertia ovina (Fabricius, 1788 or 94).

As mentioned above, about 10 of these worms were present in the bottle of *Hæmonchus contortus* from the 4th stomach of a goat.

Fam.: *HETERAKIDÆ* Railliet and Henry, 1912.
Sub-fam.: *HETERAKINÆ* Railliet and Henry, 1912.

Genus: HETERAKIS Dujardin, 1845.

Heterakis gallinæ (Gmelin, 1790) Freeborn, 1923.

The collection includes one case of this common *Heterakis* from the intestine of a fowl from Jaffa. There are about a dozen specimens, mostly females.

Genus: ASCARIDIA Dujardin, 1845.

Ascaridia lineata (Schneider, 1866), the common roundworm of the fowl is here represented by one male and several females from the intestine of a chicken. There are also three females of *Ascaridia* sp. from a fowl.

Sub-fam.: *SUBULURINÆ* Travassos, 1914.

Genus: S U B U L U R A Molin, 1860.

Subulura brumpti (Lopez Neyra, 1922) Cram, 1926.

One case only. The specimens are from the intestines of a Leghorn cock killed at Rechowoth, Jaffa district. Diagnosis is based upon the œsophagus and, in the male, pre-anal sucker and caudal papillæ. (Cram, p. 105.)

Note.—In the diagnosis and classification of the above three worms the system layed out by Cram (1927) has been used. For all the other nematodes that of Yorke and Maplestone (1926) has been made use of. Thus the family Heterakidæ is placed by the latter in the super-family Oxyuroidea, and by the former author in the super-family Ascaroidea.

Fam.: *A S C A R I D Æ* Baird, 1853.

Sub-fam.: *ASCARINÆ* (Railliet and Henry, 1912) Travassos, 1913.

Genus: P A R A S C A R I S Yorke and Maplestone, 1926.

Parascaris equorum (Goeze, 1782).

Two cases are included, one is from the stomach of a horse from Khalsa, and the other in the intestines of a horse from Jaffa. There are only three specimens in all, and all are very badly preserved.

Fam.: *F I L A R I I D Æ* (Cobbold, 1864) Claus, 1885.

Sub-fam.: *ONCHOCERCINÆ* Leiper, 1911.

Genus: O N C H O C E R C A Diesing, 1841.

Onchocerca armillata Railliet and Henry, 1909.

The most interesting specimen in the whole Palestine collection is a piece of the aorta of a bull from Jerusalem showing hard spherical fibrotic nodules from which fragments of both sexes of an onchocercine worm have been extracted. The worms are assigned to this species, which was erected by Railliet and Henry in a footnote (1909, p. 128) to contain an *Onchocerca* described and figured, but not named, by Lingard (p. 26-37, pl. IV.-X), who, in his monograph on the *Filariæ* of Indian Equidæ and Bovidæ, gives a very full and well illustrated account of these worms and the lesions they cause, but does not name them. Tuck (p. 30) gives a very detailed account of a similar infection in Malaya.

Railliet and Henry (1912, p. 117) consider that Tuck's case is the same worm as Lingard describes, i.e., *Onchocerca armillata* and also record it

from Sumatra. According to Lingard the worm usually makes sinuous galleries in the intima of the artery, but in the minority of cases he records and figures (plate X) a nodular lesion exactly like the present case.

In the material here examined the nodules are spherical and about 10 mm. greatest diameter. Both sexes of the worm lie entirely within the nodule: they do not emerge into the lumen as does *Eleophora*. The tunnel occupied by the gravid female lies within the media: there is no intimal disturbance. The media and adventitia show the same sclerosis which characterises cutaneous onchocerca nodules. In places the lining of the worms' galleries has turned black and sloughed off as inky black fragments. Both these points are as described by Lingard. These black bodies may be calcareous, but they did not give a positive reaction to silver nitrate differential staining, and there is no sign of Lingard's calcareous and fatty degeneration. The corrugation of the intima on which he remarks is only slight, but a definite induration occurs beneath each nodule on the intimal coat. Several heads of females and fragments of males were dissected out of nodules, but no male extremities were found. Both sexes cohabit the same lesion. The measurements taken correspond pretty closely with those given by Railliet and Henry (1912, pp. 117-118). The position of the vulva, the œsophageal bulb and the character of the cuticular rings also correspond with this description.

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On the Prophylactic Action of Vitamin A in Helminthiasis.

By Phyllis A. Clapham, B.Sc.

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At the suggestion of the Medical Research Council, some experiments were carried out with a view to examining the prophylactic action of Vitamin A in helminthiasis. The work has been carried out under the direction of Prof. R. T. Leiper to whom the Council made the grant which provided for this work.

Vitamin A has been shown to have protective functions of various types and in various diseases. It maintains the mucous membranes in full physiological activity, thereby preventing the bacteria which normally inhabit the lumen of the various organs from penetrating the tissues and its deficiency has been shown to lead to distinct lesions associated with certain chronic septic conditions in human beings and other animals. The lesions are of the nature of pyogenic abscesses, the most usual sites being the respiratory and urino-genital tracts (Seifried 1930, Green and Mellanby 1928 and 1930). The most obvious lesion occurs in the eye where the inhibition of the secretion of the fluid and mucus from the lachrymal and the para-ocular glands leads to xerophthalmia. Deficiency of the vitamin also has a definite pathological effect on the kidneys and the stomach wall (Fugimaki 1927) and on the hæmopoietic system (Sure and Kik, 1929).

From the point of view of helminthiasis, fewer observations are available.

Woodland in 1924 had in a vague sort of way noted that the inclusion of green food in the diet (green food being an important source of Vitamin A) rendered mice refractory to infestation with "*H. fraterna*" though he himself stresses the lack of Vitamin B and not Vitamin A which occurs more widely in green food.

Hiraishi working in Japan in 1928 on *Ascaris lumbricoides* notes the prophylactic action of the vitamin. When he fed pigs on diets low in Vitamin A he was able to infest them readily with *Ascaris lumbricoides* from a human source. The controls which had ample vitamin resisted the infestation though all the pigs took Ascaris from a pig source.

In America Ackert and his colleagues received evidence of the prophylactic action of the vitamin. They used chicks, controlling the diet carefully and infested with *Ascaridia lineata*. They established the fact that the number of worms harboured and the lengths of the worms were significantly greater in chicks which had little vitamin A in the diet.

More recently still in 1931, Foster and Cort using *Ancylostoma caninum* not only lowered the vitality and the resistance of the host to the worms but were also able to establish a partial immunity and a partial cure by simply regulating the diet with regard to its vitamin content. Their results are somewhat weakened by the fact that their diet was not only deficient in the vitamins but also in mineral salts but the general impression that one gets from the results is that in this disease also, the vitamins may play an important rôle.

Finally work in Japan by Nagoya on the larvæ of *A. caninum* in dogs, shows that the Vitamin A content of the diet affects the percentage mortality of the larvæ when given *per os* or through the skin. More larvæ were found in all the organs involved in the migration of the larvæ but he does not say if the differences are statistically sound.

Material and Technique.

The chicken was used as experimental animal in most of the experiments. They were procured as day old incubator hatched chicks and were reared under parasite-free conditions in a barn with a concrete floor. The

brooders and bedding were all sterilised before the chicks were introduced into them. At the beginning of each experiment, random samples of fæces were examined for several days but no helminth ova or protozoan cysts were ever found. The birds were therefore free from all internal parasites.

The chicks used were all cockerels of the first cross between Light Sussex and Rhode Island Reds, except in one experiment when Plymouth Rocks of both sexes were used.

The parasite chosen was *Heterakis gallinæ*. Cæca procured from a poultry dealer were used as a source of the parasite ; the helminths were collected from the cæca and cultured in Petri dishes in 1% formalin. Development occurred rapidly and infective ova were always available.

The method of infection was as follows :—

The chicks were starved for 12 hours previously. The required number of worms were isolated in a small drop of water on a slide, where they were teased up. Flour was then added slowly until a small pellet was formed and this was fed to the chick. When pushed low into the gullet of the bird, there was little danger of regurgitation. A meal was given about 2 hours later.

The dosage varied in each experiment but in all cases only the approximate dose was known. Twenty females were taken at random and teased up separately and the number of infective eggs was counted. An average was taken and the dose in terms of complete females was calculated. The average number of infective eggs found in British birds was 134.

The birds were autopsied after 24 days. This time was purely arbitrary but was decided upon as the worms are alleged to reach maturity at that time (Riley, 1931 ; Cram, 1931).

In practically all cases of feeding, infestations ensued and heavy batches of worms were obtained. But the interesting fact emerged that in all hatches there were some birds that showed a high degree of immunity and in some cases it was complete and no parasites were found at post-mortem examination of the cæca.

In one series of experiments rats were used. They were obtained at the age of two months and their fæces showed no signs of parasitic infection.

The following rations were used in the course of the experiments :—

(A) *Chicken Rations.*

(1) Adequate diet. (Ministry of Agriculture and Fisheries, 1930. Bulletin No. 7).

DRY MASH REARING RATION FOR CHICKS.
(Parts by Weight).

Age in Weeks.	1—3	3—7	7—10	10—15	15 (laying)
Maize Meal	40	45	45	50	50
Bran	20	10	10	5	5
Sussex Ground Oats	—	10	15	15	20
Coarse Middlings	20	15	15	15	20
Meat and Bone Meal	5	10	5	5	—
Dried Skim Milk	15	10	5	—	—
Extracted Soya Bean Meal ...	—	—	5	10	5
Common Salt	1	1	1	1	1
Cod Liver Oil	1	1	1	1	1
Steamed Bone Flour	—	—	2	2	2

The food was usually given as a dry mash: oyster shell and clean water were always available in separate hoppers. The mash formed an adequate diet in every way and the chicks ate as much as they desired.

(2) Diet deficient in Vitamin A. (Parts by weight.)

Middlings	30 parts
White Maize	60 ,,
Fish Meal	15 ,,
Yeast (used in Expt. (3) only)	5 ,,

This diet was not wholly deficient in Vitamin A for a small quantity was present in the middlings—the white maize and the fish meal contained none. This small amount served to prevent the onset of pneumonia and the gross lesions of A-avitaminosis and hence reduced the mortality considerably. The quantity present was not, however, sufficient for the needs of the chicks and this was evidenced by the general lack of tone and the presence of a scaly skin, lack of intra-peritoneal fat, etc.

My thanks are due to Dr. S. J. Cowell of St. Thomas's Hospital for looking over this diet and for suggesting a slight alteration in its constitution and also to Mr. E. T. Halnan of the School of Agriculture, Cambridge, for help in this respect also.

(B) *Rat Rations.*

(1) Adequate Diet.

Middlings	30 parts
Yellow Maize	30 ,,
Oats	30 ,,
Olive Oil	10 ,,
CaCO	trace
NaC1[4]	trace
Milk	one tablespoonful daily
Green Food	once weekly

(2) Diet deficient in Vitamin A.

Skim Milk Powder	30 parts
Rice Flour	40 ,,
Autolysed Yeast	15 ,,
Olive Oil	15 ,,

As with the chicken diet, this ration contained some Vitamin A—enough to prevent the heavy mortality but not enough to supply the full needs of the young rats.

Extra rations used in the course of these experiments were Vitamin D in the form of Irradiated Ergosterol. Cod Liver Oil standardised at 9 Blue units for Vitamin A ; a concentrate of Vitamin A, suggested by the Medical Research Council and made up for them by Lever Bros. This concentrate was made up in 2-minim capsules, standardised against the cod liver oil so that each capsule contained the equivalent of 5 gms. of the oil. It was dissolved in arachis oil. The fish meal used in the deficient diet was manufactured by Bicol Ltd., of Grimsby. It was made from white fish and the small amount of fat normally present was first extracted. When examined for Vitamin A, it was found to contain none.

Experiments.

(A) *To investigate the part played by Vitamin A as a prophylactic in helminthiasis.*

Experiment No. 1.—Twenty-four chicks, Plymouth Rocks of both sexes, were reared on the adequate diet (A.1) until they reached the age

of four months. They were then transferred to the diet deficient in Vitamin A (A.2) for three weeks. At the end of that time, though not showing definite xerophthalmia, they were beginning to lose tonus and to sit about in their batteries and hence it was thought that they were feeling the effects of avitaminosis. They were then fed 6 gravid and infective females of *H. gallinæ* in a flour pellet, i.e., 800 infective eggs. They were then divided into 4 groups matched for age, sex, and weight, and were fed as follows :—

(1) Deficient diet plus 1 capsule daily of Vitamin A Concentrate plus 2 minims of Irradiated Ergosterol twice weekly (Vitamin D).
(2) Deficient diet daily plus 5 gms. of Cod Liver Oil daily.
(3) Deficient diet daily plus 2 minims of Irradiated Ergosterol twice weekly.
(4) Adequate diet daily.

The worms were given on an empty crop and a meal was given two hours later. They were weighed twice weekly after fasting them for four hours. After 24 days they were autopsied and the *H. gallinæ* collected from the cæca.

The results are summarised in Table (1).

Experiment No. 2. Fifty-six sex-linked cockerels were used in this experiment. They were reared to the age of one month on the adequate diet (A.1) and then kept for two weeks on diet A.2 until a large number began to show the effects of the vitamin deficiency. They were then grouped as in Experiment (1) and fed three adult females of *H. gallinæ*, i.e. 400 infective eggs. At post-mortem examination 24 days later, results summarised in Table (2) were obtained.

It will be seen that a higher percentage survival obtains in this set of chickens. That is perhaps to be expected as these chicks were younger and therefore more susceptible to infestation than the older birds.

Turning to the growth of the worms, the mean lengths show an increase of 2-4 mms. over those in the previous experiment while the standard deviation from the mean is somewhat less. These two points can probably be attributed to the youth of the birds as well.

Experiment No. 3. Forty-four sex-linked cockerels were used in this experiment—a repetition of Experiment No. 1. The only difference was the addition of 5 per cent. untreated baker's yeast to the deficient

Group.	No. of birds.	Diet.	Total No. of worms.		% Survival.	Max. No. in one bird.		Min. No. in one bird.		Mean.			Mean Length in mms.		Standard Deviation.	
			♂	♀		♂	♀	♂	♀	♂	♀		♂	♀	♂	♀
1	6	Capsule of Concentrate	94	134	3·54	47	56	0	0	15·5	20·16	35·66	5·37	5·97	1·16	1·45
2	6	Cod Liver Oil	222	259	7·41	84	87	7	11	36·6	43·3	79·9	5·51	6·06	0·72	1·12
3	6	Deficient for Vitamin A	47	69	2·2	15	22	0	0	7·8	11·5	19·3	5·46	5·31	1·49	1·48
4	6	Adequate diet	171	198	7·46	67	65	0	0	28·3	32·86	61·16	6·68	8·00	1·00	1·10

TABLE I.

Group.	No. of birds.	Diet.	Total No. of worms.		% Survival.	Max. No. in one bird.		Min. No. in one bird.		Mean.			Mean Length in mms.		Standard Deviation.	
			♂	♀		♂	♀	♂	♀	♂	♀		♂	♀	♂	♀
1	14	Vitamin Concentrate	807	860	29·8	109	112	24	30	57·5	61·3	118·8	7·59	8·38	0·53	0·89
2	14	Cod Liver Oil	929	889	32·5	126	144	31	30	66·3	63·5	129·8	7·82	9·24	0·44	0·49
3	14	Deficient Vitamin A	623	753	24·6	86	103	10	13	44·5	53·8	98·3	8·12	9·48	0·50	0·77
4	14	Adequate diet	282	427	14·5	62	58	0	0	14·0	30·5	44·5	6·97	8·02	0·38	0·53

TABLE II.

basal diet (diet A.2). This was added because it was felt that Vitamin B was scanty. The results were striking. Firstly with regard to numbers; there were still no significant difference between the groups. Those in Group (2)—i.e. those receiving cod liver oil—harboured less worms than usual. Only one bird proved entirely immune and it belonged to this group. The percentage survival has risen in all the other groups—not much in Group (1) which received Vitamin A in the form of the concentrate but there was a 25 per cent. rise in Groups (3) and (4).

Group.	No. of birds.	Diet.	Total No. of worms.		% Survival	Max. No. in one bird.		Min. No. in one bird.		Mean.	
			♂	♀		♂	♀	♂	♀	♂	♀
1	11	Vitamin Concentrate	750	696	33·2	250	222	0	2	68·19	63·27
										131·46	
2	11	Cod Liver Oil	447	382	18·9	154	123	0	0	40·64	34·72
										75·36	
3	11	Deficient Vitamin A	828	770	36·3	188	147	7	6	75·27	70·00
										145·27	
4	11	Adequate diet	467	493	21·8	79	68	1	0	42·45	44·81
										87·26	

TABLE III.

Next with regard to the length—all the worms harboured were not measured this time. A large sample was taken from each group and no significant difference was found. The results tallied with the earlier ones. The writer had looked for a general increase in length as Ackert (1931) had evidence that yeast contains a factor, influencing the growth in length of *Ascaridia lineata*. No such effect was found here.

In all these experiments bi-weekly weighings were made and it was found that the birds belonging to Group (3) were usually on an average 10 per cent. lighter at the end of the experiment than the other groups, though all groups started equal. These differences were not statistically significant but they are suggestive of the fundamental deficiency of the diet.

(B) *To investigate the occurrence of Host Specificity in* Heterakis gallinæ.

Heterakis gallinæ, as already noted in a previous paper dealing with the life history, has been recorded as occurring in a large variety of game and domestic birds. In spite of the wide distribution enjoyed by some of these hosts, it is worth while to consider the possibility of host specificity on the part of the parasite. Such a state of affairs is not unknown among helminths. Take for example the case of *Ascaris lumbricoides* and "*A. suis*." These two forms are identical morphologically but physiologically there is a distinct difference, which shows itself in the fact that *A. lumbricoides* (human strain) is not infective to the pig while "*A. suis*" will not infect a human. However, this difference is not absolute and Hiraishi, working in Japan (1928), was able to overcome it by paying attention to the diet. He made up two diets, each having the same nutritional value but differing in their vitamin content and he fed these to two groups of pigs. Later he fed eggs of *Ascaris lumbricoides* (human strain). His results showed that no infestation had occurred in the pigs which had had adequate Vitamin A while in the other group a decided infestation was obvious. The conditions were identical except for the vitamin concentration of the diet and hence this must have been the deciding factor. He inferred, therefore, that the vitality had been so lowered by the action of the deficient diet, that an infestation had been able to establish itself.

There are other examples of the production of biological races among helminth parasites chief among which are the dwarf tapeworm of man and rodents—*Hymenolepis nana* and "*H. murina*": *Ancylostoma caninum* and *A. braziliense* of canines and felines: and *Strongyloides stercoralis* of man, a strain of which morphologically indistinguishable shows a host specificity for various Carnivores.

Such a condition is not recorded for *H. gallinæ*, but it was thought that the problem was worthy of investigation. With this end in view, Heterakis was collected from the cæca of the pheasant (*Phasianus colchicus*), guinea fowl (*Numida meleagris*) and the turkey (*Meleagris gallopavo*). Chicks which had been fed the diet (A.2) deficient in Vitamin A were fed eggs collected from these hosts, while the controls were fed the same diet with the addition of 1 capsule of Vitamin A daily.

At a post-mortem 24 days later, all the chicks were found to be infested, and there was no significant difference between the numbers obtained from each group.

The results are summarised in the accompanying table. The numbers and percentages given represent the averages obtained from four chicks. In each case the birds fed the additional vitamin show a slightly lower percentage but it is not significant at any time.

	Strains.		
Chicks fed 133 eggs (1 adult female)	Turkey	Pheasant	Guinea Fowl.
Gp. (1). Fed diet A.2 only	31 (=23·3%)	42 (=31·6%)	47 (=35·3%)
Gp. (2). Fed diet A.2 with additional ration of Vitamin A ...	29 (=21·8%)	24 (=18·0%)	36 (=27·0%)

We may therefore assume that *H. gallinæ* from these hosts is normally infective to the chickens and this infectivity is not significantly affected by the Vitamin A concentration of the diet.

(C) *To investigate the effect of A-avitaminosis on the hatching and survival of* Parascaris equorum *in rats.*

Albino rats were obtained at the age of 7 weeks for these experiments and fæces examination showed them to be helminth-free. They were divided into two groups and fed diets B.1 and B.2. At the age of four months experimentation began and at this time there was a distinct difference in the general vitality and size of the animals. The early symptoms could be seen in those of Group B.2.

Each had its own control. They were fed as follows:—(1) 5,000; (2) 10,000; (3) 20,000; (4) 50,000 infective eggs of *P. equorum* and were killed by shooting 72 hours later. The feeding method was to starve them overnight and to feed the eggs mixed with a small quantity of flour so as to form a pellet. At post-mortem examination the liver, lungs, trachea and stomach were examined by means of Baermann extractions. When the rats were opened up, it was obvious that migration had taken place as the lungs were hæmorrhagic. In all cases the stomach contents proved to be negative and attention was concentrated

mainly on the lungs. The smaller doses proved ineffective in producing the pneumonia and finally 50,000 eggs was chosen as the standard dose.

After extracting in a Baermann apparatus not all the larvæ were counted. The fluid was run off and shaken and a small quantity was examined for larvæ. All the larvæ were counted in this sample and the total number estimated. An average of eight experiments was taken with the following results expressed as percentages :—

(1) Rats fed a diet adequate for Vitamin A ... 17·6% of the number administered.

(2) „ „ „ deficient „ „ „ ... 38·2% of the number administered.

In all cases the variation was less than 8 per cent. These differences are statistically significant.

Apart from these figures, some interesting facts emerge. The fæces of these animals were examined during eight hours after infestation. A large number of eggs were passed after the first hour. The number steadily increased up to five or six hours, after which it fell rapidly. Hence for all observations fæces passed after five hours were used. Random samples were examined in saline and all the eggs counted.

In Group B.1 (fed adequate vitamin) for every 100 empty shells there were 242 shells containing active larvæ and 32 free larvæ. In Group B.2 (fed deficient vitamin) for every 100 empty shells there were 84 shells with larvæ and 35 free larvæ. These free larvæ had probably hatched in the intestine, for it was found that large numbers of larvæ hatched in the stool after it was passed. The infertile and undeveloped eggs were ignored.

The numbers calculated in percentages are :—Group (1), 21·8 per cent. hatched ; Group (2), 46·0 per cent. hatched.

Percentages obtained from ...	Liver and Lungs.	Stool.
Group B.1	17·6	21·8
Group B.2	38·2	46·0

They bear a close relationship to the numbers obtained from the larvæ found in the lungs after 72 hours and seem to show that a fair idea of hatching and survival can be obtained from fæcal examinations.

Roughly 20 per cent. of the larvæ that were hatched are unaccounted for but one must bear in mind this fact that some would still be in the blood stream, while others might have reached abnormal positions such as the kidneys or the body cavity. Hence we may say that in this case the larger number of those larvæ which hatch in the small intestine survive and undertake the migration through the body.

Some of the eggs which passed through the intestine without hatching were fed to rats and produced lesions in the lungs and hence they were viable.

Another interesting fact which emerged from these experiments is the speed of the migration. In the group receiving deficient vitamin the rate was speeded up. Of the total number of larvæ found in any single rat there was always a higher percentage found in the lungs of those receiving deficient vitamin while in the other group a somewhat lower percentage was found in the lungs after 72 hours and rather more in the liver. That is to say that pneumonia was more acute in Group B.2. Sections showed the typical picture—an exudate into the alveoli and respiratory passages and a marked inflammation.

To summarise these results we have definite evidence that the hatching and survival of *Parascaris equorum* in albino rats is significantly affected by the Vitamin A content of the diet. In rats fed deficient vitamin larger numbers of larvæ hatch and migrate and the rate of development of the larvæ is more rapid than in rats fed adequate vitamin.

Discussion of Results.

The results obtained by the present writer on the whole are not in agreement with those of previous writers. Ackert found that he could produce significant differences in both numbers and length of *Ascaridia lineata* obtained from chickens using diets of known composition of Vitamin A.

Hiraishi found that he could overcome the natural resistance of pigs to the human strain of *Ascaris lumbricoides* by controlling the Vitamin A content of the diet. A rather similar result was obtained by Foster and Cort, working on the dog hookworm *A. caninum.* They were able to establish an immunity and to bring about a partial cure by controlling the Vitamin concentration of the diet.

The results catalogued in this paper can bear no such definite interpretation. The experiments which have been carried out are directly comparable to those of Ackert and of Hiraishi, yet contrary results obtain. The reason for this may lie in the life cycle of the parasite concerned. *Ascaridia lineata* is for three weeks a tissue parasite whereas *H. gallinæ* is always an occupant of the lumen of the intestine and of the cæca. The writer hesitates to put forward any reason for these differences, however, without further evidence.

It is obvious that the age of the host affects the percentage survival of the parasites ingested for in the first experiments when young adults were used, a much lower survival rate obtained in all groups. The last two experiments are directly comparable as far as technique is concerned but the results are different. Group (4) in both cases shows some degree of resistance ; results from Group (2) are strikingly different in the two experiments.

The only conclusion that can be drawn from these results is that in the case of *H. gallinæ* infestation of the chicken, the Vitamin A content of the diet does not directly affect the resistance of the host.

Turning to Ascariasis in rats, we have evidence that the vitamin content of the diet significantly affects the fate of the helminths both as regards numbers and rate of development. The lower the amount administered, the greater the survival rate and the more rapid the development. Hence one tends to get a more acute pneumonia while in rats fed more vitamin and given an equal dose of eggs the pneumonic symptoms tend to be more dispersed and spread over a longer time besides being less severe.

Some Notes on the Viability of Heterakis Eggs.

(1) *Eggs which have passed through the body unhatched.*

A large number of eggs pass through the body unhatched and can be found apparently unchanged in the fæces from two hours after infestation. After giving a heavy dose of eggs to a chick, the droppings were collected and the eggs concentrated in a small quantity. This was mixed with the ordinary mash and fed to two chicks which were examined after 36 days. In this experiment also the brooders were not cleaned up for 48 hours after infestation, thus allowing the chicks every possibility of picking up eggs.

At post-mortem examination it was found that they harboured a light infestation of *H. gallinæ*, and we can therefore assume that eggs which have once passed through the gut are still viable and liable to bring about an infestation if ingested again.

(2) *Eggs which have passed through an Earthworm.*

It is usually assumed that earthworms are a possible method of the dissemination of helminth eggs. In order to investigate this problem a large number of Heterakis eggs were mixed with sterile soil. Worms which had previously been fed on blotting paper to empty the gut were introduced into this soil and allowed to feed for two days. They were then transferred to blotting paper again and the droppings collected and these were fed to chickens.

Other worms given similar treatment were washed in saline and brushed with a firm brush to remove the mucus from the cuticle and hence to remove any eggs which might be adhering to the mucus. These earthworms were fed to chicks.

After 36 days all the chicks were killed and it was found that they harboured a definite infestation of *H. gallinæ*, thus showing that eggs which have passed through the gut of an earthworm or are still within the gut are viable and liable to produce an infestation.

Summary.

(1) Experiments have been conducted to investigate the part played by Vitamin A in the prophylaxis of helminthiasis. *Heterakis gallinæ* in the chicken and *Parascaris equorum* in albino rats were used.

(2) The evidence tends to show that in Heterakis infestation the vitamin content of the diet has no effect on the course of the infestation. In Ascariasis in rats, however, the vitamin affects significantly the hatching, survival and rate of development of the larvæ within the host.

(3) The writer can put forward no reason for this difference except to suggest that it may lie in the life history of the parasites. Considering her own results and those of previous workers as cited in the introductory part of this paper, it seems obvious that the vitamin is effective in cases where the parasite comes into close contact with the host tissues.

(4) Some notes on the viability of eggs are added.

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Significant Factors in the Plerocercoid Environment of *Diphyllobothrium latum* (Linn.)

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The problem of rearing endozoic helminthes in artificial media is likely to remain one of the major problems of helminthology until a means of domesticating such helminthes has been found, a means of divorcing them from their host environment and studying them in *vitro*. Until such a technique has been established, the numerous physiological problems of helminth parasitism cannot be attacked with much hope of success. The evaluation, therefore, of the influence of each significant variable in the helminth environment upon the viability and upon the behaviour of the helminth, is a question of more than mere academic interest, since a technique of helminth cultivation can only be founded upon a series of such evaluations.

Diphyllobothrium latum was selected for the present study chiefly because it can be obtained in quantity in this area. It is nevertheless one of the most suitable cestodes for experimental purposes. Its life cycle is fairly completely known, its transition from larval stage to adult maturity is gradual, its life-cycle includes both poikilothermic and homoiothermic hosts, and it can be readily reared to maturity in laboratory animals such as the cat or dog.

This cestode, or a species identical morphologically with it, is endemic in the Canadian province of Manitoba. Its incidence in man in this area is undoubtedly considerable, although no figures are available. It is common in dogs along the shore of certain lakes ; an examination of 500 sled dogs in the vicinity of Lake Winnipeg made by my colleague Dr. A. Bajkov in the summer of 1931 revealed an incidence of 85 per cent. It occurs to some extent in the cats of the larger towns ; an examination of 70 vagrant cats in the city of Winnipeg during the winter

1930-31 showed a percentage infection of 4·5. It occurs also in the Black Bear (*Ursus americanus* Pallas) and the Mink (*Putorius vison* Scherber) although it may be doubted whether the suggestion by Vergeer (1928) that wild mammals constitute an important reservoir of infection can be substantiated.

The normal environment of the plerocercoid stage in Manitoba is the epaxonic musculature, and, to a lesser extent, the hypaxonic musculature of certain fishes ; more rarely, they occur on the peritoneal surface of the alimentary tract. The species of fishes which act as hosts in Manitoba waters are, in order of incident infection, *Esox lucius* Linn., *Perca flavescens* (Mitchill), *Lucioperca vitreum* (Mitchill), *Lota lota maculosa* Le Sueur, and *Lucioperca canadense* (Smitt).

These appear to be the only piscine hosts of the plerocercoid stage of *Diphyllobothrium latum* in Manitoba. Since however these fishes are all fish-eating, there is a gap in our knowledge of the helminth life-cycle between the procercoid stage, known to occur in *Diaptomus oregonensis*, and the plerocercoid stage in the fish. The suggestion sometimes made that very young pike or perch are plankton feeders and so would become infected with plerocercoids which would then be passed on to larger fishes which fed upon the young pike or perch, is unsatisfactory, since there appears to be, in the intramuscular plerocercoid, no mechanism which would enable it to bore through the alimentary tract of the large fish and penetrate into the muscles. In fact, there appear to be no grounds whatever for assuming that plerocercoid stages in the muscle tissue of one fish can pass from the gut of a second fish and into its musculature.

In the southern area of Lake Winnipeg, possibly the most heavily infected lake in the province, the incidence of infection during the summer and autumn of 1931 ranged form 85 per cent. in *Esox lucius*, varying somewhat from month to month, to as low as 7 per cent. in *Lucioperca canadense*, the mean percentage of infection among the whole five species being 28 per cent. It may be noted that the southern end of Lake Winnipeg receives the Red River which is the main sewer for untreated sewage from the city of Winnipeg and although this city is 40 miles from the lake, such fishes as the pickerel and the pike migrate up and down the river nearly as far as the sewer inlets.

With the exception of *Lota* all the host fishes of *Diphyllobothrium latum* in Manitoba are marketed for human consumption. *Lucioperca vitreum* is marketed extensively in Canada and the United States under such names as "pickerel," "yellows" and "wall-eyed pike," and Manitoba is the chief source of supply for the North American continent. This fish is marketed in an unfrozen condition, and as the fishing is carried on in sub-zero weather through holes in the ice, special care is taken to prevent the fish from freezing during transmission to market.

These host fishes also form the principal food of the large numbers of sledge dogs around the larger lakes.

The intramuscular plerocercoid phase, unlike the majority of other *Diphyllobothrium* plerocercoids, is not surrounded by a host-secreted membrane, but lies slowly expanding and contracting between the host muscle fibres, its invaginated bothrial extremity being situated cephalad to the longitudinal axis of the fish. The width between successive nyocommata in a 5-year old pike is 5-8 mm., and plerocercoids less than 8 mm. in length lie parallel to the fibres and are straight; plerocercoids between 8-14 mm. in length usually have the anterior third turned back upon the body in hook-like fashion; the longest larvæ may be considerably twisted and may extend into adjacent myotomes. The larva is cylindrical or clavate, in outline, 5-20 mm. by 1-1·5 mm. in dimensions, glistening opaque white in colour, slightly wrinkled, and invaginated at either extremity, the bothrial invagination being indicated by a short groove. Larvæ from Canadian fishes have been accurately figured by Vergeer (1929).

If such plerocercoids be fed to kittens and recovered, they are found in the small intestine; after 6 hours they are 3-7 inches from the pylorus, after 12 hours they are 7-16 inches from it, and after 24 hours they are as far as 30 inches from it. In shape and behaviour they are in marked contrast to the intramuscular forms. They are smooth, translucent, expanded, ribbon-like; the scolex is fully everted and vigorously mobile. The abothrial extremity is truncated as if a portion of the larva had been digested or broken off, and the larva is less in average length than the intramuscular form. The scolex is sharply pointed but after 24 hours it is rounded like that of the adult worm and adheres, although

feebly, to the mucosa of the host. There is no evidence of proglottisation until the fifth day after initial infection.

Since the term *plerocercoid* implies that stage in the dibothriocephaloid life-cycle which intervenes between the loss of the caudal appendage and the commencement of proglottisation, and which is characterised by the possession of a scolex similar to that of the adult, and by the absence of recognisable genitalia, *Diphyllobothrium latum* may be said to have an intramuscular plerocercoid phase and an enteric plerocercoid phase, the latter being spent within the mammalian host gut and passing gradually into the condition of proglottisation and incipient genitalia development which characterises the immature adult.

The transition from one phase to another is a transition from one set of physico-chemical values favourable to the intramuscular phase to another set of values, favourable to the enteric phase ; a transition from the wide range of low temperature values of the fish to the narrow range of relatively high temperature values of the mammal ; from the low electrolyte concentration of fish muscle juice to the relatively high concentration of mammalian intestinal juice ; during this transition, the larva is exposed to varying values of hydrogen ion concentration and osmotic pressure, and to gastric and duodenal enzyme attack.

The influence of varying values of these factors is thus of considerable biological interest.

The Influence of Temperature.

Published observations upon the temperature tolerance of larval *Diphyllobothrium latum* are scanty.

According to Kjava (1913), larvæ within infected fishes are killed by 48 hours exposure of such fishes to an external temperature of —9°C. and remain viable within the fish at environmental temperatures between 3-50°C. Seno, Kitagawa and Iwamato (1925) record the presence of three dead pleroceroids in specimens of *Oncorhynchus masou* which had been exposed for five days to an external temperature of —18—19°C. Magath and Essex (1931) record the death of plerocercoids in *Lucioperca vitreum* after exposure for 24 hours to an external temperature of —15°C. and the probable death of plerocercoids in similar fishes which had been

exposed for 88, 40 and 16 hours to an external temperature of —10°C. Having established by a series of preliminary observations that plerocercoids of *Diphyllobothrium latum* will remain alive with a host fish for at least twelve hours, so long as the fish does not putrefy, when the host is exposed to external temperatures between zero and mammalian blood temperature, the following observations were made to ascertain the influence, upon plerocercoid viability, of temperature values outside this range.

Sub-zero temperatures were obtained by using ice-saline mixtures in an insulated chamber. Temperatures between 20° and 60°C. were obtained in a thermostat. These temperatures were not absolutely constant, the sub-zero values having a fluctuation of ±2° and the super-zero temperatures having a fluctuation of ±0·5° C.

The larvæ were taken from *Esox lucius* and *Lucioperca vitreum* within an hour of removal from the nets, the fish being then 3-10 hours dead. Fish muscle juice was impracticable as a medium owing to its rapid putrefaction and owing to the large amount that would be required. On the other hand, preliminary observations had shown that plerocercoids will remain viable and active for at least twelve hours in 0·2 molar NaCl at temperatures between zero and 40° C. As a matter of fact, the later experiments with electrolyte concentrations indicated that concentrations of 0·1 molar NaCl or 0·2 molar $CaCl_2$ would have been even more favourable.

However, a series of experiments was carried out with naked larvæ in 0·2 molar NaCl, and a series with larvæ left *in situ* each within a small piece of fish muscle, each larva being separated from the atmosphere by a sheet of muscle fibres not exceeding 2 mm. in thickness.

The procedure, briefly stated, was to place 10-20 larvæ in the chamber at a selected temperature, and by hourly inspections to establish the death point as lying between minimum and maximum hourly values. The experiment was then repeated, the inspection intervals being reduced to 15 minutes when the minimum hourly value was reached. Larvæ vary considerably in their susceptibility to temperature so that the survivial times given below represent mean values of a number of larval survival times.

The viability of larvæ was judged by their response to tactile stimulation

with a camel's hair pencil when in molar saline at 38° C., the frozen imbedded larvæ having first been thawed out in the solution. Death was presumed when over a period of several hours in warm saline the larvæ remained irresponsive, became expanded and flaccid, and commenced to disintegrate. The number of larvæ used rendered impracticable any confirmation of larval death by feeding to dogs, even if such test could be relied upon.

The following values of survival time were obtained :—

	Time in hours.	
T° C.	NaCl.	Muscle.
55·0	0	—
54·0	0·25	—
52·0	1·0	0·75
50·0	—	2·6
48·0	3·6	—
46·0	—	6·3
44·0	30·0	—
42·0	33·0	—
38·0	36·0	12+
20·0	60·0	—
0	24+	—
— 2·8	—	12+
— 3·0	1+	—
— 3·5	—	4·5
— 5·0	—	1·16
— 7·0	—	0·91
— 8·0	0	—
—10·0	—	0·5

The maximum and minimum lethal temperatures for larvæ in 0·2 NaCl appear to approximate to 55° and —8° C. respectively. At temperatures below zero and above 47° the larvæ become instantaneously immobile but recover after a few minutes and may show response to stimulation for several hours.

Discussion.

The survival time values of naked plerocercoids when in 0·2 molar NaCl and exposed to temperature values ranging from 20° to 44° C.,

are fitted by a curve which in general conformation is a typical thermo-metabolic curve, such as is found to represent the influence of temperature upon organic development and viability generally. That is to say, the curve is an equilateral hyperbola whose reciprocal is a straight line cutting the X-axis at a point indicative of the threshold of development or of the minimum lethal temperature. In this case, the minimum lethal temperature indicated is —8·3° C., which agrees fairly well with the experimentally found value of —8°C.

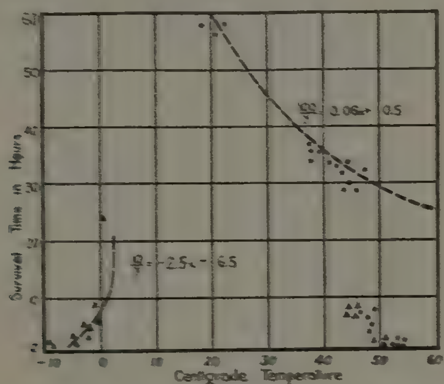

Fig. 1. The influence of temperature upon the viability of plerocercoid larvæ of Diphyllobothrium latum. ● denotes naked larvæ in NaCl; ▲ denotes larvæ within a coating of muscle fibres.

At temperatures above 44° C., however, and possibly at temperatures below 20° C., the curve departs widely from the hyperbolic type, the survival values at temperatures between 44° and 54° C. being very much lower than they should be if the curve were hyperbolic throughout.

The survival time values of intramuscular larvæ at temperatures above 44° C. approximate sufficiently closely to the values for naked larvæ as to suggest that the film of muscle around each larva is not so efficient an insulator as to interfere with the influence of the external temperature values.

The internal temperature, however, of a piece of pike muscle approximating in thickness to the commercial fillet, that is 1·5–2·5 cms. alters

only slowly when the muscle is placed in an abnormally low or high external temperature, the rise or fall apparently following the Compound Interest Law, and larvæ imbedded in such a thickness of muscle, although killed when the external temperature exceeds 55° C. or falls below —8° C., are killed probably by such factors as desiccation and bacterial disintegration, or by the mechanical pressure of frozen muscle fibres, rather than by the actual internal temperature values of the muscle.

The survival time values given for imbedded larvæ at temperatures between zero and —8° C. are probably too high, the frozen muscle coating acting to some extent as an insulator and retarding the restrictive influence of low temperature values upon larval viability.

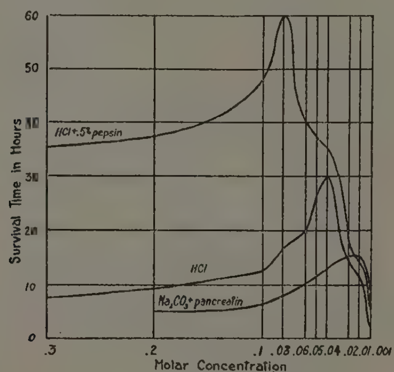

Fig. 2. The influence of HCl, HCl+ pepsin, of Na_2Co_3+ pancreatin upon the viability of plerocercoids of *Diphyllobothrium latum* at 38° C.

The gradual increase in the length of the survival time as temperatures fall from 55°-20°, and the decrease in length of survival time as temperatures fall from zero to —8°, suggest that the optimal range of survival time values occurs at temperatures between 38° and zero centigrade. At what range of values between these points the survival time value is at its maximum is a point of some importance. The mean summer body temperature of *Esox lucius* in Lake Winnipeg during 1931 was found to be 20·3° C. If it could be shown that the optimum

range of survival time values lay on either side of such a mean temperature value, and that survival time diminished as the host body temperature departed from the mean summer value, some light would be thrown upon the low numerical incidence of plerocercoids in the individual fish, a question which will be alluded to later in the paper.

THE INFLUENCE OF DIGESTIVE JUICES.

The hydrochloric acid content of pure canine gastric juice varies between 0·45–0·58 per cent. (Rosemann, 1920), that is to say between 125 and 160 degrees of acidity, one degree of acidity being equivalent to a 0·001 molar concentration of HCl. During protein digestion, however, the acidity does not exceed 60 degrees. The pepsin content varies from 0·1–0·5 per cent.

The alkalinity of pure duodenal juice from the dog, expressed as Na_2CO_3 varies around 0·42 per cent., the NaCl content around 0·5 per cent.; the pancreatin content is around 0·2 per cent.

Plerocercoids dissected cleanly from freshly caught *Esox lucius* were exposed, at a temperature of 38° C., to concentrations of HCl from 0·3—0·001 molar, to similar concentrations each containing 0·5 per cent. of pepsin, to concentrations of Na_2CO_3 from 0·2—0·001 molar, and to similar concentrations containing 3 per cent. of pancreatin.

Observations were made at hourly intervals and the mean values of survival time were as follows:—

Molar conc.	HCl.	HCl + Pepsin.	Na_2CO_3.	Na_2CO_3+ Pancreatin.
0·3	8	36	—	—
0·2	—	—	∠ 2	5·3
0·1	13	48	∠ 2	7·3
0·08	17	60	—	—
0·06	20	40	—	—
0·05	—	—	∠ 2	12·0
0·04	30	36	—	—
0·02	15	18	—	—
0·01	12	15	∠ 2	15·5
0·001	∠ 3	∠ 5	∠ 2	∠ 5·5

The plerocercoids were able to tolerate to some extent all the concentrations of HCl in the molar range employed, the tolerance being greatest in 0·04. The presence of pepsin in the HCl concentration increased the tolerance period in all concentrations. At all concentrations of HCl + pepsin that occur normally in the canine stomach, the plerocercoid would apparently remain viable for a time period exceeding the time it would be exposed to peptic digestion.

The tolerance of plerocercoids for Na_2CO_3 is low, the specimens disintegrating within two hours in all the concentrations employed. If, however, pancreatin be present, tolerance is greater. It may be concluded that plerocercoids can tolerate all values of Na_2CO_3 between 0·2 and 0·001 molar, if pancreatin be present, for a length of time exceeding the time they would normally be exposed to pancreatic digestion.

No conclusions were arrived at with regard to the physico-chemical nature of the resistence of plerocercoids towards digestive enzymes. Scott (1913) has suggested that resistance of cysticerci to gastric digestion is brought about by muscular contraction of the body surface. Hamill (1906), however, has demonstrated in *Ascaris* the presence of an anti-tryptic substance in the tissue juices, thus confirming the earlier assertion of Weinland (1903) and opposing the view of Dastre and Stassano (1904) that the substance was anti-kinasic. The possibility of anti-peptic and anti-trypstic anti-bodies in cestode larvæ deserves investigation.

The Influence of Physiological Salines.

When naked plerocercoids are placed in a physiological saline, such as Ringer-Locke solution, with the glucose content omitted so as to reduce the possibility of bacterial or fungal interference, they become active, and at 38° C. will live between 36-48 hours. If the solution be changed frequently and be kept at laboratory temperatures around 20° C. the plerocercoids may live for a week. In appearance and behaviour they resemble plerocercoids lying *in situ* in the intestine of a kitten.

Such a saline is essentially a solution of NaCl (0·15 molar) plus a little KCl (0·003 molar), $CaCl_2$ (0·002 molar) and $NaHCO_3$ (0·002 molar).

The relative influence on plerocercoid activity of the respective components of such a saline is thus a matter of interest.

Plerocercoids were exposed, therefore, to a series of concentrations of these salts ranging from 0·001-0·2 molar, at a temperature of 38° C.; observations being made at intervals of an hour.

The survival time values observed were as follows, the data representing mean times expressed in hours.

	Molar Concentrations.				
	0·2	0·1	0·05	0·01	0·001
NaCl	30	40	70	4	2
$CaCl_2$	60	28	12	3	2
KCl	3·5	3·5	18	4·5	1
$NaHCO_3$ ...	8	12	15	1·25	0·5

The conclusion may be drawn that plerocercoids of *Diphyllobothrium latum* can stand exposure to the component salts of Ringer-Locke solution, when in concentrations between 0·001 and 0·2 molar, for a period of time varying with the concentration and with the chemical structure of the salt. The tolerance is greatest in 0·05 molar solutions except in the case of $CaCl_2$, where tolerance increases with molar concentration within the range of concentrations employed. Toleration is least at values of 0·001 molar.

It may be added that at 38° C. the tolerance of larvæ in 0·2-0·1 NaCl is about the same as in Ringer-Locke solution but at temperatures around 20° C. it is greater, larvæ living in unchanged NaCl for as long as 13 days, larvæ in Ringer-Locke never living longer than a week. The addition of low concentrations of KCl, $CaCl_2$ and $NaHCO_3$ to decimolar NaCl do not appear to improve it as a medium for these plerocercoids.

Discussion.

The viability of plerocercoid larvæ of *Diphyllobothrium latum* in molar concentrations of the electrolytes—HCl, Na_2CO_3, $NaHCO_3$, NaCl, KCl, $CaCl_2$—varies with temperature, with the nature of the electrolyte, and with the molar concentration, but the behaviour of such larvæ in molar concentrations of the range 0·2-0·001 is essentially similar, irrespective of the nature of the electrolyte.

There is (1) a preliminary period of activity marked by irregular expansion and contraction of the animal ; this is followed by (2) a period of latent mobility, the larva moving only when touched and undergoing a slow, gradual muscular contraction, its shape changing from cylindrical to discoid, its body becoming deeply and transversely wrinkled ; there is (3) a final period of endosmotic immobility, the larva becoming fully expanded, flaccid, transparent, irresponsive to touch, and rapidly disintegrating.

These periods vary in relative duration. In NaCl, KCl, $CaCl_2$ the

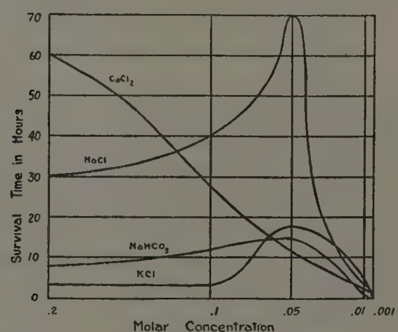

Fig. 3. The influence of NaCl, $CaCl_2$, KC1 and $HaHCO_3$ upon the viability of plerocercoids of *Diphyllobothrium latum* at 38° C.

activity is comparatively prolonged, the succeeding periods being passed through rapidly. The active larva may have the median region expanded, the bothrial and abothrial extremities remaining contracted, or may be fully expanded and smooth, the scolex fully everted, and may resemble closely in appearance and activity the enteric plerocercoid phase. In HCl the activity period is short—although it can be prolonged by the presence of pepsin—and the larva is never fully expanded and never has the scolex everted ; the second period is comparatively long.

Scott (1913), commenting on the behaviour of *Cysticercus pisiformis* in artificial gastric juice, suggests that the non-eversion of the scolex, and the deep contraction of the surrounding tissue, protects the vital

region of the larva from digestion even though the bladder becomes digested. That such contraction may protect the larva from digestion seems to be suggested by the rapid disintegration of plerocercoids in HCl or HCl + pepsin after a preliminary period of a few hours in 0·2 molar NaCl whose effect seems to be that of weakening the muscular contractibility of the larva and rendering it more permeable to acid penetration. The difficulties experienced by some workers in infecting mammalian hosts with *Diphyllobothrium* plerocercoids is explicable if the larvæ had been exposed previously to physiological saline. (c/f Le Bas, 1924.)

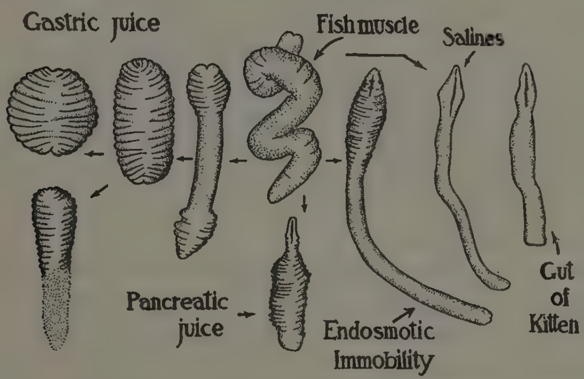

Fig. 4. The appearance of plerocercoids of *Diphyllobothrium latum* in various media.

In some cases the contraction period is passed through very rapidly or omitted, the larva passing from activity into endosmotic immobility and rapid disintegration. Such appears to be the case in concentrations of Na_2CO_3, in concentrations of electrolytes below 0·001, in distilled water, in solutions exposed to the minimum and maximum lethal temperatures, and in $CaCl_2$ after a relative long period of activity.

The periods may overlap. The anterior half of the larva may undergo expansion and contraction, whilst the posterior half is undergoing deep, progressive contraction. In $NaHCO_3$ + pancreatin the scolex is expanding and contracting whilst the body of the larve is deeply contracted. In HCl the abothrial extremity of the larva may undergo endosmotic

disintegration whilst the preceding region is in a state of latent mobility, such latent mobility being retained even when only 2 mm. of undisintegrated tissue remain.

It must be emphasised that the behaviour of plerocercoids in electrolyte concentrations is not altogether explicable as due to differences in the osmotic gradient between the *milieu interieur* and the surrounding medium. The absence of distortion, twisting, body-bloating during the activity and latent immobility periods, and the similarity of larval behaviour within each concentration of a relatively wide range, suggest that the behaviour may be influenced by a specific effect upon muscular irritability of ions or of whole molecules passing from the medium into the larva.

The similarity of behaviour in electrolytes with the Cl' anion would suggest that the larval surface is permeable to Cl' and that this ion provides one such stimulus to muscular irritability. It must be admitted, however, that in a number of experiments in which 50 larvæ were kept for 24 hours in a known molar concentration of NaCl no evidence could be obtained by subsequent colorimetric titration of the solution that any NaCl had been abstracted.

There seems a possibility that Cl' can pass through the body surface of a cestode and raise the internal Cl' content, even above the value of the Cl' content of the medium, the internal and external values being then held in a Donnan equilibrium.

According to Schopfer (1926) the NaCl content of *Cysticercus tenuicollis* is 7·2 per cent. (circ. 1·5 molar), that is to say approximately 15 times the NaCl content of the mammalian host blood. The NaCl content, again, of the nematode, *Ascaris megalocephala*, according to the same author, is 1-1·2 per cent., approximately twice that of the host intestinal juice. This worker suggests that although the body surface of the *Cysticercus* is permeable to NaCl there is a Donnan equilibrium between the internal Cl' content and the lower Cl' content of the medium.

On the other hand, since in dilutions of sheep's serum +NaCl the *Cysticercus* gains or loses weight, presumably through absorption or through loss of water, and since *Ascaris* responds to changes in osmotic pressure of the horse's intestinal juice by gaining or losing weight, the internal osmotic pressure of the worm may not be determined solely by the NaCl content.

The freezing point depression of the internal fluid of adult *Diphyllobothrium latum* is —1·09° C., according to Vialli (1923), indicating an internal osmotic pressure of approximately 13 atmospheres, nearly twice that of the intestinal juice of the mammalian host where the freezing point depression is —0·62° C.

Observation, in the present study, of plerocercoids in a range of cane sugar solutions whose osmotic pressures were equal to those of a range of 0·2-0·001 molar NaCl, appeared to indicate that the internal osmotic pressure of the plerocercoid is between three and four atmospheres.

The Cl' content of fresh *Esox* muscle, expressed as the mean value of ten Van Slyke determinations, is 0·035 per cent., a value which agrees fairly well with the value of 0·032 per cent. given by Dische (1926) for European specimens of *Esox*. If the osmotic pressure of the muscle juice is determined by the Cl' content, it is lower than that within the plerocercoid. On the other hand, if, as appears to be the case, the freezing point of muscle juice in *Esox* approximates to that of its blood, where $\Delta = 0{\cdot}519$-$0{\cdot}530$, Dekhuyzen (1905), the osmotic pressure of the muscle juice will be higher than that within the plerocercoid.

The depression of the activity period which occurs eventually when plerocercoids are exposed to pure concentrations of an electrolyte can only be explained, since the duration of the activity period differs with each halogen electrolyte used, as due to a specific action of the kation of neutral salts, or possibly the whole molecule of acids and alkalies, either upon the larval body surface or upon its internal colloids. Such specific toxicity of acids, alkalies and certain kations such as Ca'' towards animal membranes is well established. Dispersal of the surface membrane, or ionisation of the internal proteids, will induce an inrush of water and a disintegration of the larval tissues.

The effect, therefore, of pure solutions of electrolytes upon plerocercoid viability and behaviour is eventually the same, although its culmination is slower in arriving, as that of pure water, of weak electrolyte concentrations, or of lethal temperatures.

The question as to whether disintegration of a pre-maturation stage of a cestode is a normal procedure in the appropriate host, is one to which as yet no answer can be given. There is no direct evidence that intra-

muscular plerocercoids of *Diphyllobothrium latum* disintegrate eventually in their habitat or that they can be destroyed by host tissue reactions. The following figures, however, appear to suggest that the life of the intramuscular phase is limited and that there is variation in host susceptibility.

	Examined.	Per cent. infected.	Mean number larvæ per infected fish.
Esox lucius	578	69	2·3
Perca flavescens	211	30	2·1
Lucioperca vitreum ...	267	25	1·7
Lucioperca canadense ...	1453	7	1·2
Lota lota maculosa ...	80	11	1·1

The fish were caught within a three-mile radius of Gimli, on Lake Winnipeg, during July and August; the feeding habits of all these species are similar, all feeding upon smaller fishes.

Among fishes caught during the same period in Lake Winnipegosis, an area of lower infection than Lake Winnipeg, the mean number of larvæ per infected pike was 2·7, the mean number per infected pickerel (L. *vitreum*) was 1·2. Among 26 infected pickerel from Canadian waters, examined by Vergeer (1929), the mean number of larvæ per fish was 2·03.

The mean number of larvæ per fish appears too low to be explained by lack of opportunity of the host to ingest sufficient numbers of infected small fishes and suggests that either all these fishes are resistant to plerocercoid establishment or that the duration of life of the intramuscular plerocercoid is comparatively short.

Mention was made earlier in this paper of the truncated extremity of all plerocercoids recovered from infected kittens. A similar truncation can be produced in plerocercoids by exposing them for several hours in 0·04 molar HCl + 0·5 per cent. pepsin at 38° C. and then transferring them to decimolar NaCl and it is possible that disintegration of the abothrial extremity of the plerocercoid occurs during the period it spends in the mammalian host stomach, such disintegration being analogous to that of the bladder of cysticerci when exposed to mammalian gastric digestion.

The viability of the adult *Diphyllobothrium latum* in its mammalian host has not been established with certainty. Riley (1919), Leiper (1928) and others have brought forward evidence that the worm may live in man for several years, but the evidence has been adversely criticised by Ward (1930).

In the few dibothriocephaloid cestodes whose life cycle has been studied, the period of adult life appears to be brief. Thus Rosen (1918) asserts that *Eubothrium crassum* in *Trutta lacustris* matures in this host at the end of March and is expelled from the host gut in a disintegrated condition about the end of August, and that *Triænophorus tricuspidatus* in the gut of *Esox lucius* in Europe matures about the end of February and is expelled towards the end of June.

In the case of *Triænophorus tricuspidatus*, which is common in *Esox lucius* in Manitoba, some unpublished observations by a colleague, appear to suggest that this worm matures in the pike in late January and has been expelled by the end of May.

On the other hand, the possibility of perennialism among dibothriocephaloid cestodes cannot be dismissed. There is reason to believe, for example, that *Eubothrium oncorhynchi* of the Pacific Salmon can live in its final host for several years (Wardle, 1932).

Diphyllobothrium is exceptional among dibothriocephaloid genera in utilising homoiothermic final hosts and it may be exceptional in being perennial in such hosts.

In dogs, *Diphyllobothrium latum* appears to be readily expelled after maturity is reached, particularly if there be co-infestation with *Toxacara canis*.

It may be added that there is considerable variation in the susceptibility of dogs and cats towards *Diphyllobothrium latum*, some animals being almost impossible to infect, even when care is taken to prevent the plerocercoids being crushed between the teeth of the animal ; other animals, again, are most readily and easily infected ; the state of hunger of the animal does not appear to influence its susceptibility.

It is possible, therefore, that a more detailed study of the influence of molar concentrations of single electrolytes, of mixed electrolytes and of electrolyte concentrations containing colloids, under varying

conditions of temperature, hydrogen ion concentration, and so on, might provide an explanation of many hitherto unexplained phenomena of specificity among cestodes.

Summary of Conclusions.

1. Plerocercoid larval stages of *Diphyllobothrium latum* (Linn.), in 0·2 molar sodium chloride can tolerate temperature values between —8° and 55° C., the survival time varying from 15 minutes at 54° C. to 60 hours at 20° C., and diminishing gradually from 24+ hours at zero to non-survival at —8° C. The optimum range of values lies between 38° and —2·8° C. It is suggested that the median point of the optimum range approximates to the mean summer temperature of the host fish and that variations in temperature from this mean point restrict the survival time value of the intramuscular plerocercoid phase.

2. They can tolerate concentrations of HCl between 0·3 and 0·001 molar if 0·5 per cent. of pepsin be present, and concentrations of Na_2CO_3 between 0·2 and 0·001 molar, when 3 per cent. of pancreatin is present, for the length of time they would normally be subject to canine or human gastric and duodenal digestion.

3. They can tolerate the component salts of Ringer-Locke solution when in concentrations between 0·2 and 0·001 molar for periods of time varying with the concentration and with the chemical structure of the salt. The addition of other electrolytes to decimolar NaCl does not increase its value as a medium for the larvæ.

4. The behaviour of plerocercoids in electrolyte solutions of 0·2—0·001 molar concentration is essentially similar, and initial period of activity being followed by a period of contraction and latent mobility—which, however, may be omitted—passing into a period of endosmotic immobility and disintegration. It is suggested that activity is stimulated by the influence of absorbed Cl' upon muscular irritability and that depression of activity is induced by absorption of the kation of neutral salts or of the whole molecule of acids or alkalies.

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Anguillulina graminophila n.sp., a Nematode causing Galls on the Leaves of Fine Bent-grass.

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Introduction.

About the middle of July, 1932, red galls were discovered on blades of grass in a meadow at Winches Farm, St. Albans, by Mr. W. A. Macdonald, who drew the attention of the writer to the presence of nematodes within them. Subsequent search on this and an adjoining meadow proved that the galls were widely distributed, on the same kind of grass, over the whole area, whilst some days later similar galls were found on a bank by the side of the main road from St. Albans to Hatfield. The grass was identified as *Agrostis tenuis* Sibth. (*Agrostis vulgaris* With.), popularly called Fine Bent-grass.

From a study of the records of grass leaf-galls given in Houard (1908-13) it was found that Karsch (1880), Schlechtendal (1885), and Rübsaamen (1890) had described galls on the leaves of *Agrostis vulgaris*. Consultation of these papers showed that the galls found in Germany were red in colour, were commonly found to occur towards the bases of the leaves and had about the same dimensions as those under observation. Only Schlechtendal of the three early recorders stated that the worms within the galls belong to the genus *Tylenchus*.

Of the known species of *Anguillulina*, two produce galls on grass leaves, namely *A. graminis* (Hardy, 1850) Goodey, 1932, which is the causal organism of leaf-galls on species of *Festuca*, and *A. tumefaciens* (Cobb, 1932), which gives rise to galls on leaves, stems and flower-heads of a lawn grass, *Cynodon transvaalensis*, and was described by the late Dr. N. A. Cobb in a paper which appeared about three weeks before his death. It was a matter of interest, therefore, to determine whether

the worms responsible for the galls on *Agrostis tenuis* belonged to either of these species. Examination of the adults from freshly gathered galls soon showed that the parasites belong to a hitherto undescribed species of *Anguillulina*, for which the specific name *graminophila* is proposed.

Occurence of *Anguillulina graminophila* n. sp.

Material has been plentiful, with the result that it has been possible to carry out detailed observations, not only on the anatomy of the adults, but also on the course of larval development ; thus throwing light on the life-history of the parasite, and on the pathology of the galls. Dissection of numerous red galls during July and August revealed the presence in most cases of adults and some eggs. The number of adults per gall varies considerably from two or three to 11 or 12 of each sex. Older galls of a blackish purple colour were found to contain adults, eggs and larvæ in various stages of growth from short 1st stage forms to long slender 4th stage pre-adult larvæ, which constitute the infective stage. A few young galls, greenish yellow in colour were dissected and contained immature adults which had still to undergo their final ecdysis before passing into the adult condition. They were of about the same size as the 4th stage larvæ from the old galls, but their genitalia were slightly more advanced in growth. It was clear, therefore, that the 4th stage larvæ must have been the ones to set up the new galls.

Morphology.

Dimensions :—*Female* : length, 1·6 mm. to 2·5 mm. ; stylet, 0·012 mm. ; $\alpha = 33\text{–}26, \beta = 11\text{–}10, \gamma = 26\text{–}23, V = 86\%\text{–}90\%$. *Male* : length, 1·4 mm. to 2 mm. ; spicules, 0·044 mm. ; gubernaculum, 0·018 mm. to 0·02 mm. ; $\alpha = 43\text{–}30$; $\beta = 8{\cdot}1\text{–}7{\cdot}5, \gamma = 26\text{–}20$.

In both sexes the body tapers in front and behind. The cuticle carries fine transverse striations. The head, which is distinctly offset by constriction, is button-shaped with well rounded sides and is apparently made up of six rounded lips. The cuticle of the head is rather thicker than on the rest of the body and the thickening is carried back on to the front end of the body to a distance about equal to the depth of the head. The stylet has the usual structure ; an anterior conical half joining to a posterior cylindrical half, the base of which carries three rather large rounded thickenings. The œsophagus is of the normal Anguillulina

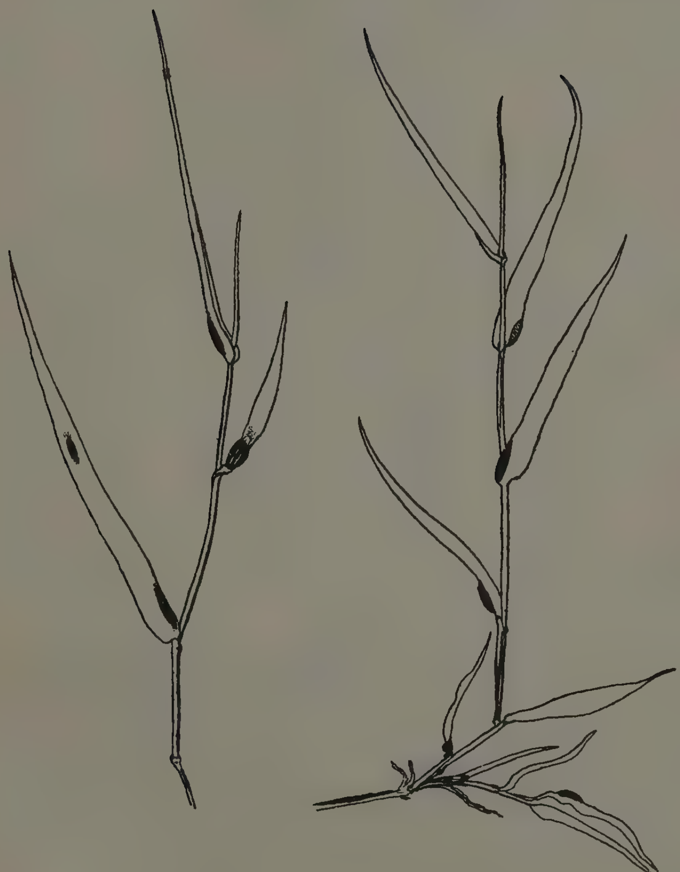


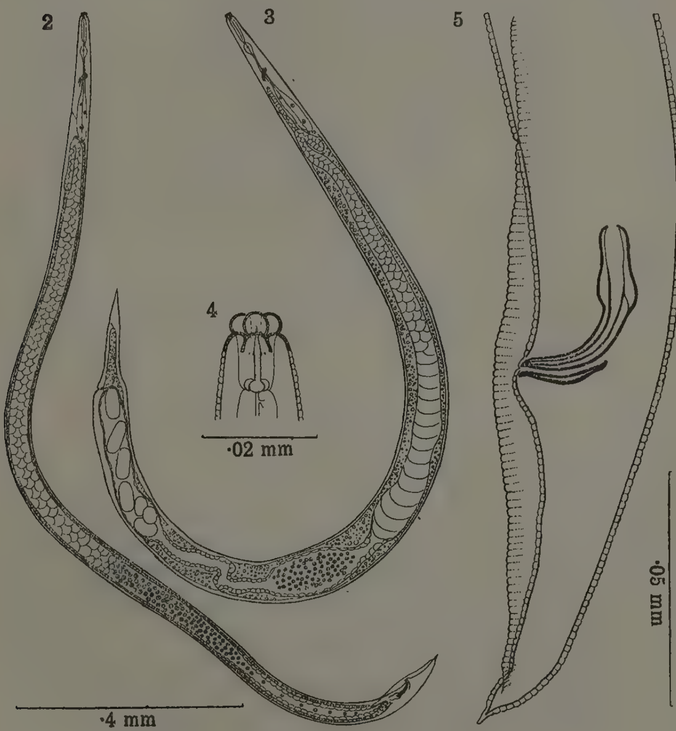
Fig. 1. Drawing of two portions of *Agrostis tenuis* Sibth. (Fine Bent-grass), showing galls as heavily shaded areas. Natural size.

type with a median ellipsoidal bulb containing small crescentic thickenings of the lumen. This bulb is connected by a comparatively long neck region to the posterior glandular portion which is rather long and contains the three œsophageal gland cells. The intestine, rectum and anus are normal and call for no special description. The nerve ring crosses the œsophagus a short distance behind the median bulb, and the excretory pore occurs in the vicinity of the posterior swelling of the œsophagus.

Female.—The body narrows rather steeply immediately behind the vulva, and then gently tapers towards the tip of the tail where it becomes conical and ends in a sharp point. The anus and rectum are fairly easily seen. The vulva has the form of a broad ventral slit, and its anterior rounded lip is rather prominent. The short, thick-walled vagina leads almost horizontally into the uterus and the post-vulval uterine sac, which is present in so many species of this genus, is here entirely absent or is represented by the merest local thickening of the end wall of the uterus. The uterus is a comparatively long tube capable of accommodating as many as 14 eggs at a time. Following a slight constriction of its wall, the anterior end expands into an oval or ellipsoidal receptaculum seminis, which is often densely packed with round spermatozoa. Another constriction in the wall separates this region from the ovary, which from this point runs forwards in the body and, becoming gradually narrower, reaches as far as the œsophagus, where it is often doubly reflexed on itself for a short distance.

Male.—Adults of this sex are, on the whole, slenderer than females and, when teased out from fresh red galls are generally very motile. The body in many cases narrows down somewhat in the region of the vas deferens as a consequence of which the bursa stands out rather prominently. The anus is situated on a rounded protuberance, and the tail then tapers gently to the terminus which is conical and sharply pointed. The caudal wings forming the bursa arise considerably in front of the anus at a point, in most cases, about equal to the length of the tail. They do not enclose the tip of the tail, but join the body rather steeply immediately in front of the tip. Each wing is comparatively deep, so that the whole bursa is voluminous. The free edge of each wing is finely crenate and caudal papillæ are absent. The spicules are paired and comparatively large, measuring about 44μ long. Each has a fairly sharp point, and

then gradually increases in width as we approach the head region which makes up a little more than one-third the total length of the organ and has a somewhat elongated appearance very similar to that exhibited by the spicules of *A. dipsaci* and *A. radicicola.* As seen in lateral aspect,



Anguillulina graminophila n. sp.

Figs. 2 & 3. Male and female respectively under low magnification to show general structure ; lateral view. Lower scale on left.

Fig. 4. Head end highly magnified in lateral view.

Fig. 5. Male tail highly magnified in lateral view ; showing one caudal wing, one spicule and gubernaculum. Lower scale on right.

its widest part occurs close to the shaft of the spicule following which it becomes slightly concave on either side, and then swells out a little at the front end; the two sides curving inwards. An inner strengthening rib supports each side and runs from the point into the head region; extending further on the ventral than on the dorsal side. The gubernaculum is a rather stout structure with pointed ends, and is thickest in the middle where it is hollow. The testis is single and its blind end, which lies close to the posterior end of the œsophagus, is doubly reflexed for a short distance. It gradually increases in width as we approach the tail and swells out for a short distance a little more than half-way down the body into a vescicular seminalis. Posterior to this it becomes tubular with fairly stout walls, and is here the vas deferens.

Eggs.—The eggs are cylindrical with rounded ends and measure 0·085 mm. to 0·095 mm. long by 0·035 mm. to 0·04 mm. wide. They are laid in a many-celled stage of segmentation.

Larvæ.—First stage larvæ measure from 0·4 mm. to 0·45 mm. in length. Stylet, œsophagus, intestine, rectum and anus are all fairly easily seen. The genital primordium appears as a small clear area about half-way down the intestine on the ventral side. The tail tapers gently behind the anus, but is suddenly steep on the dorsal side at the terminus. Four ecdyses are passed before the adult stage is reached, and three of these take place within the gall cavities. The first moult occurs when the larvæ are about 0·54 mm. long. The second stage larvæ grow till they reach about 0·65 mm. long, when the second moult takes place. The third stage larvæ become longer and the genital primordium elongates somewhat, leading to the third ecdysis when the larvæ have reached a length of about 0·9 mm. to 1·1 mm. Still further growth takes place till the larvæ measure from 1·2 mm. to 1·43 mm. in length and have now reached the infective stage. Close examination under fairly high powers enables one to differentiate the sexes at this stage; the females being recognised by the presence of the rudiment of the vulva a short way in front of the anus, and by the fact that the tail region is more gently tapering than in the males where it is rather abruptly conical from the anus backwards. These pre-adult larvæ are generally very active when teased out from old galls in water. They are able to withstand desiccation, since if the water in which they are swimming is allowed to evaporate they become active again when re-moistened. For how long a time they

can remain viable in dried galls is a point which it has been impossible, as yet, to determine.

Life History.

The fourth stage, pre-adult larvæ escape from old galls and invade the tissues of young leaves, whilst these are still held within leaf sheaths. As a result of the action of the parasites on the tissues of the host, galls are formed in which the worms, after undergoing their fourth and final moult, become young adults. After further growth in length and thickness has taken place, pairing occurs, and the females produce an abundance of eggs. The latter hatch and give rise to larvæ which pass through the development already indicated till they reach the long slender pre-adult infective condition. In developing to this 4th stage whilst still within the gall the larvæ differ from those of *Anguillulina tritici*, *A. agrostis* and *A. graminis*, in all of which larval development within the gall ceases at the 2nd stage, which then constitutes the infective stage. In this respect the new species resembles *A. dipsaci* and *A. balsamophila* in both of which pre-adult larvæ form the infective stage.

Symptoms.

The general health and appearance of the grass did not appear to be adversely affected by the presence of galls on the leaves. The galls occur most frequently at or close to the base of the leaves, but occasionally are found half-way down the blade or even close to the tip. Two or more are often found side by side, especially at a leaf base and, becoming united, cause considerable local swelling. Leaves carrying such large galls appear to be somewhat shorter than unaffected leaves. Most of the galls are single and are fusiform in shape ; varying in length from 1 or 2 mm. to 10 or 15 mm., by 1 to 2 mm. in width. In the young condition they are greenish yellow in colour ; they then become reddish purple and finally purplish black. They are quite firm or even hard to the touch.

Pathology.

On examining a simple gall under the microscope it is found, as a rule, to involve the tissues of the leaf between and on either side of two parallel veins. Compound galls involve at least three veins. A narrow and fairly deep groove runs down the length of the gall on the upper surface

of the leaf, whilst the underside is usually rounded and may show a flattish median longitudinal ridge.

A transverse section across a simple gall shows the structure figured in fig. 6. It can be seen that the tissues of the normal leaf, shown on either side of the drawing, have a typical ridge and furrow appearance ; the ridges running along the veins and the furrows lying between them. The first thing that strikes one in the galled portion is its relatively large size compared with the normal leaf tissues. There is not only individual cell hypertrophy, but cell multiplication of all the tissues involved including epidermis, mesophyll and vascular bundles. The longitudinal cleft along the upper surface probably represents the furrow normally lying between two ridges ; its depth being determined by the extent of the swelling up of the affected leaf tissues on either side of it. The mesophyll cells are individually much enlarged and, for the most part, are somewhat oblong in shape. They occur in from five to nine rows, lying more or less parallel to the rounded curvature of the gall. Many of these cells evidently break down and so give rise to cavities within the gall in which the parasites lie freely. The enlarged mesophyll cells are very granular in appearance, and those abutting on the gall cavities are not so green as the deeper lying ones, but are rather yellow in colour. The nuclei of many of the cells are large and round ; a condition often presented by the nuclei of cells in galled tissues, e.g., those in the cortical cells of galls on barley roots caused by *Anguillulina radicicola*, recently described by the writer (1932).

The red colour of the galls is due to the presence of a purplish-red cell sap occurring in the mesophyll cells immediately underlying the epidermis and occasionally in cells of the latter also.

Host. Agrostis palustris Huds., form *Agrostis tenuis* Sibth. (*A. vulgaris* With.) (Fine Bent-grass.) In classifying the grass in this manner the writer follows the 7th edition of Bentham and Hooker, 1930, in which it is considered merely as a form of the very variable *Agrostis palustris* Huds. (Fiorin grass), which was *A. alba* L., of earlier editions of this standard work.

In Armstrong's "British Grasses," 2nd edition, 1921, *A. vulgaris* is considered as possibly a small variety of *A. alba*, and the writer's identification of the grass found in the meadows at Winches Farm is based on the details given by Armstrong, namely, small size of the plant, short

ligule and the teeth on the keel of the lower empty glume occurring only in the upper half. A comparison was made in respect to these characters on the so-called *A. stolonifera*, and the two forms were found to differ in these details.

Occurrence.—In meadows and on a road-side bank at Winches Farm, St. Albans, Herts., England. Earlier records show that it also occurs in Germany.

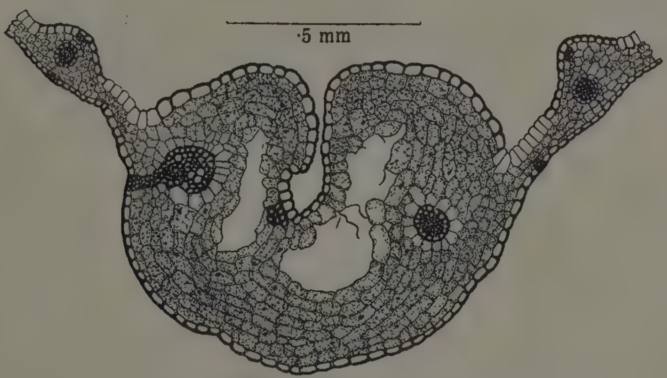


Fig. 6. Transverse section of a simple gall on a leaf of *Agrostis tenuis* under low magnification. Epidermal, vascular and sclerenchyma cells are represented with thickened walls. The parasites occur in the cavities formed amongst the mesophyll cells.

Systematic Position.

In considering the systematic position of the worms a comparison is called for with two nearly related species of *Anguillulina* causing galls on grass leaves; namely *A. graminis* (Hardy, 1850) Goodey, 1932, and *A. tumefaciens* (Cobb, 1932).

1. *A. graminis.* This species gives rise to galls on the leaves of *Festuca dumetorum*, *F. duriuscula*, *F. ovina* and *F. rubra.* The new species differs from it in the following important features: (i.) The adult female of *A. graminis* possesses a fairly long post-vulval uterine sac which is entirely absent or merely vestigial in *A. graminophila.* (ii.) The ovary is considerably reflexed as a single loop in *A. graminis*, but in

A. graminophila is doubly reflexed in two short loops. (iii.) The bursa is smaller in *A. graminis* than in *A. graminophila*. (iv.) The spicules in *A. graminis* are narrower at the head end than in the shaft, whereas in *A. graminophila* they are widest at the head. (v.) The infective stage in *A. graminis* is the 2nd stage larva, measuring 0·67 mm. to 0·7 mm. long, whilst in *A. graminophila* it is the 4th stage pre-adult larva measuring 1·2 mm. to 1·43 mm.

2. *A. tumefaciens*. This species causes galls on leaves, stems and flower-heads of *Cynodon transvaalensis* (?). It somewhat resembles *A. graminophila* in having the ovary doubly reflexed anteriorly and in the general shape of the bursa, as shown in Cobb's fig. 3. The two species differ, however, in the following principal features.

(i.) Adults of *A. tumefaciens* are smaller than those of *A. graminophila*; females being 1·4 mm. long, as compared with 1·6 mm. to 2·5 mm., and males 1·2 mm., as compared with 1·4 mm. to 2 mm. in *A. graminophila*.

(ii.) The uterus of *A. tumefaciens* accommodates only one egg at a time, whereas there may be as many as 14 eggs at a time in that of *A. graminophila*.

(iii.) A post-vulval uterine sac is present in *A. tumefaciens*, and has a length of about 4% of the body, i.e., about 58μ, whereas it is absent or vestigial in *A. graminophila*.

(iv.) The spicules of *A. tumefaciens*, as figured by Cobb, have an entirely different shape from those of *A. graminophila*; being broadest in the middle, lacking an elongated, expanded head and in having rounded points.

Unfortunately, Cobb gave no particulars as to which is the infective larval stage in the case of *A. tumefaciens*, consequently the two species cannot be compared in this respect.

In view of the above considerations it is necessary to erect a new species for the reception of the worms, and they are accordingly named *Anguillulina graminophila* n. sp.

Association of the Fungus, *Dilophospora alopecuri*.

In his very interesting investigations on *Dilophospora alopecuri* (Fr.) Fr., on wheat, spelt, and rye, Atanasoff (1925) discovered the co-parasitism of this fungus with the nematode, *Anguillulina tritici*, and concluded,

as a result of numerous experiments, that the association between fungus and nematode is obligatory for the fungus, i.e., he could not set up the symptoms of disease due to *D. alopecuri* except through the agency of *A. tritici*.

The obligatory character of this association has since been disproved by Schaffnit and Wieben (1928), who succeeded in infecting wheat with *D. alopecuri*, apart from the nematode *A. tritici*. The fact remains, however, that under natural conditions the two pathogens are intimately associated with each other as Atanasoff found. The co-parasitism of the two was observed by the writer in 1928 and 1929 on the experimental plot devoted to *A. tritici* on wheat at Winches Farm, and is probably of wide occurrence in nature.

Atanasoff further tabulated the various cereals and grasses known as hosts of the fungus, and in a parallel column set out whether they were also hosts of *A. tritici* or of some other species of this genus. Amongst the grasses listed in his table is *Agrostis vulgaris*, on which *D. alopecuri* was noted by Kirchner in 1906, whilst Schlechtendal (1885) is cited for the occurrence of nematode galls on this host. He suggested that there exists a relationship between the two pathogens probably in all the various hosts.

The purpose of the following remarks is to put on record the fact that the writer has observed a definite association between *D. alopecuri* and *A. graminophila* on *Agrostis tenuis* (*A. vulgaris*). A few almost black galls were found early in August on grass leaves and were opened in the expectation that nematodes would be found within them and surprise was felt when, instead of worms, the thickened walls were found to contain spherical bodies (pycnidia), which on breaking open liberated masses of pycnospores of *D. alopecuri*. At this time note was merely taken of the fact that disease due to the fungus was present on this particular species of grass. At the end of August a black gall was opened and was found to contain pycnidia of the fungus and, in addition, two or three 4th stage larvæ of *A. graminophila* attached to the cuticle of which were some pycnospores of *D. alopecuri*. A few days later a multiple gall in the young, yellow-green stage of development, was opened and contained several 4th stage larvæ of the nematode, a few of which were thickly covered with pycnospores and secondary spores of the fungus.

These observations clearly establish a definite relationship between the nematode and the fungus, and show that the spores of the latter may become attached to the bodies of the former. Whether the association is obligatory, for the fungus cannot, of course, be proved without further observations and experiments, but it is evident that the fungus can be distributed by the nematode larvæ, and it is at least feasible that it may infect the tissues of the host where they have been damaged by the nematode.

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A Preliminary Note on *Bilharzia margrebowiei*, a new Parasite of Ruminants and possibly of Man in Northern Rhodesia.

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Introduction.

During the examination of numerous specimens of Bilharzia collected from domesticated and wild ruminants in this Territory a species of bloodfluke, closely related to the vector of intestinal bilharziasis in the Far East, was observed. For this species the name *Bilharzia margrebowiei* sp. nov. is designated in honour of my wife, Dr. Margaret Gregor Leroux (née Bowie), for the self sacrificing way in which she has assisted me in the past.

The study of the literature, dealing with bilharziasis in Africa, suggests that the eggs of this species have possibly been met with in human stools in Central Africa.

The specimens, on which the diagnosis of this species is based, were collected from cattle, a zebra, lechwe kobs (*Cobus leche* Gray), reedbuck (*Redunca arundinum* Bodd.), pukus (*Cobus vardoni* Livingstone) a blue wildebeest or brindled gnu (*Connochaetes taurinus* Burch.), a situtunga or water kudu (*Tragelaphus spekei selousi* Roth.), and a roan antelope (*Hippotragus equinus* Desm.).

Bilharzia margrebowiei was invariably found along with other species of Bilharzia in the various hosts mentioned above. In the majority of hosts it was found to inhabit the radicles of the portal vein along with *Bilharzia spindalis*. In a few cases the hosts were also infested with *Bilharzia mattheei* (Veglia and Le Roux, 1929).

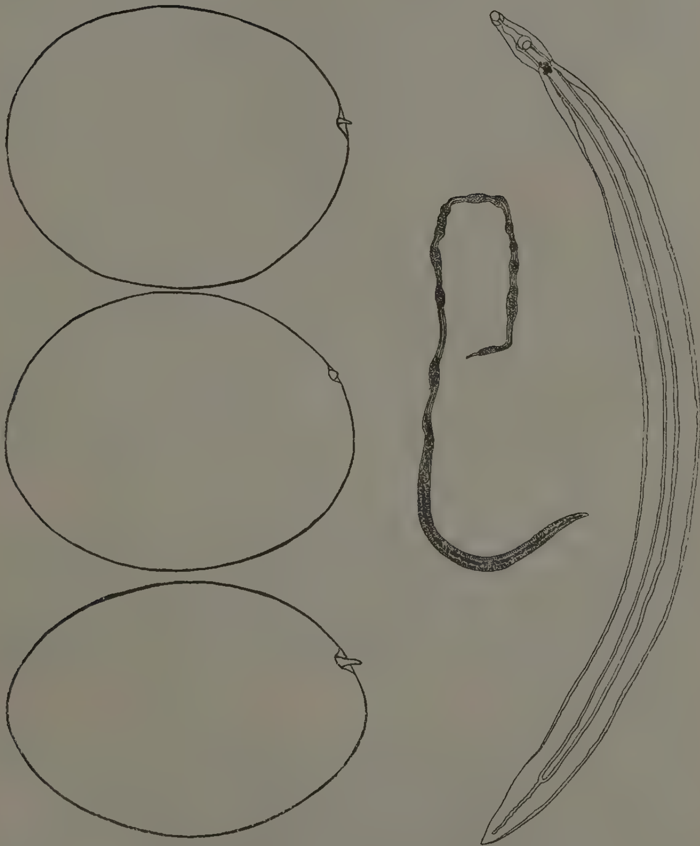
Bilharzia spindalis (Montgomery, 1906) and *Bilharzia mattheei* are common parasites of sheep, cattle and antelopes in this locality. The latter has been collected from man and the baboon *Papio porcarius* in Southern Rhodesia by Blackie (1932).

Morphology of *Bilharzia margrebowiei* sp. nov.

Male.—12 to 18 mm. long by 0·857 mm. to 1·254 mm. wide. Cuticle armed with bosses and spines dorsally and spines ventrally. Inner surfaces of suckers provided with spines. Oral sucker subterminal, with a lateral diameter of 200μ to 240μ. Ventral sucker pedunculated and situated at a variable distance, according to the state of contraction of the individual, from the oral sucker. Testes, usually four more rarely five in number, measure approximately 176μ by 160μ. The seminal vesicle in front of the testes and variable in dimensions depending on the sexual activity of the individual at the time of collection. In some specimens the measurements of this organ were 176μ by 112μ. The intestinal cæca unite very late as figured in the accompanying drawing.

Female.—14 mm. to 20 mm. long by 270μ to 320μ wide. Inner surfaces of both suckers and posterior portion of body armed with spines. Ovary, somewhat pear-shaped, 688μ long by 225μ wide and situated at equator in well preserved specimens. In specimens collected from slightly decomposed viscera the ovary was post-equatorial (See Fig., p. 59.) Uterus long and containing numerous eggs arranged in clumps as figured. Eggs (See Fig.), practically oval, 60μ by 45μ to 70μ by 42μ, provided with a small subterminal knob, rudimentary or well developed spine as figured. Vitellaria occupy the posterior half of the worm in freshly collected and well preserved specimens. These organs are, in specimens collected from semi-decomposed viscera, confined to less than the posterior half, owing to the undue elongation of the ante-ovarial half of the worms.

It cannot be over-emphasized that the various measurements of all trematodes but more especially the members of the genus *Bilharzia*, are subject to enormous variations brought about by methods of collection, modes of preservation and the state of putrefaction of the viscera of the host at the time of collection.



Eggs and Adults of *Bilharzia margrebowiei* n.sp.

Discussion.

The species described above is larger than any of the other locally collected members of the genus. A casual examination of a female would suggest that the eggs are unarmed and that they are probably identical with the eggs observed in human stools in the Belgian Congo by Walkiers (1928). This author did not recover any worms but designated the name *Schistosoma faradjei* for the species responsible for the eggs seen by him.

Cawston (1930) records that he had seen a bilharzia egg, resembling that of *Bilharzia japonica*, in the stools of a native from Northern Rhodesia. It would now seem probable that he was dealing with an egg of this species.

The males of *B. margrebowiei* differ from those of *B. japonica* in that they have four to five testes and the cuticle on the dorsal surface is provided with numerous cuticular bosses armed with spines. The presence and the degree of development of these cuticular bosses are greatly affected by the state of vitality of the specimens at the time of preservation. By allowing specimens of *B. spindalis*, *B. mattheei*, *B. indica* and of this species to die in normal saline before preservation in 10 per cent. Formalin, it was observed that cuticular bosses were absent and that the cuticle appeared perfectly smooth. These bosses were likewise absent in specimens collected from semi-decomposed viscera.

The differentiation of the males of the various species of *Bilharzia*, parasitizing domesticated animals in Africa, is a most tedious undertaking. The males of *B. mattheei*, *B. bovis*, *B. curassoni* and *B. margrebowiei* are very much alike morphologically especially when young specimens of the larger species are to be differentiated from full-grown specimens of the smaller species.

From a study of the various species collected locally I must conclude that the identification of any one species is only possible when a male, with a matured female in his gynæcophoric canal, is submitted.

An identification, based on the shape and size of the eggs observed in the fæces of the host, is unreliable when it is remembered that the eggs of *B. mattheei*, *B. bovis*, *B. curassoni* are very much alike. The eggs of *B. indica* are not too readily distinguished from those of the species just mentioned. It is in human cases quite possible to mistake the eggs of *B. mattheei* for those of *B. hæmatobia* and those of *B. margrebowiei* for those of *B. japonica*.

It may be mentioned here that I have obtained from a zebra and wild ruminants a species which has tentatively been identified as *Bilharzia indica* (Montgomery, 1906) and it would now seem probable that either this species or *Bilharzia curassoni* (Brumpt, 1931), or both, may in Central Africa be responsible for the human cases of intestinal bilharziasis where a terminal-spined egg has been observed without the urinary bladder being involved.

It is of interest to note that the species of Bilharzia now known from Africa are :—

1. *Bilharzia hæmatobia* from man.
2. *Bilharzia mansoni* from man.
3. *Bilharzia bovis* from sheep, cattle and man (?).
4. *Bilharzia mattheei* from sheep, goats, cattle, antelopes, baboons, man and the grey monkey (experimental infection).
5. *Bilharzia spindalis* from sheep, cattle, horse, antelopes and man (Porter, 1929).
6. *Bilharzia spindalis* var. *africana* from man.
7. *Bilharzia curassoni* from cattle.
8. *Bilharzia lombarti* (Syn. *Schistosoma rodhaini* Brumpt, 1931) from an experimentally infected mouse.
9. *Bilharzia indica* from zebra and antelopes.
10. *Bilharzia margrebowiei* from cattle, etc.

The type specimens and co-types of *Bilharzia margrebowiei* sp. nov. have been deposited in the Helminthological Collection of the Department of Helminthology at the London School of Hygiene and Tropical Medicine.

Acknowledgements.

The writer takes this opportunity of acknowledging his indebtedness to Prof. J. H. Ashworth and Prof. R. T. Leiper for working facilities in their respective departments in Edinburgh and in London, and for having had access to their collections of helminthological material which proved most helpful in the identification of the helminths collected in Northern Rhodesia. He further wishes to express his gratitude to Prof. E. Brumpt for specimens of *Bilharzia bovis*.

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Helminthology in its Applications to Marine Fisheries.*

By T. Southwell, D.Sc.

Historical.—Our knowledge of the cestode parasites of marine fishes is due almost entirely to the work of Linton in North America, Shipley, Herdman and Hornell in Indian waters, Zschokke and Beauchamp in Europe. We know nothing regarding the tapeworms found in fishes in South America, round the coast of Africa, or in the Arctic ; and our knowledge of those found in the Far East is limited to descriptions of about ten species by Yoshida. It will, therefore, be apparent that there still remain large areas to be investigated.

Geographical distribution.—The parasites are doubtless cosmopolitan. As, however, Plagiostomes are much more abundant in tropical than in temperate waters, it naturally follows that cestodes are much more frequently found in tropical areas. There are several parasites common to India, Europe and America, but quite a number of species appear to be limited in distribution to specific areas, and to particular hosts.

Adult worms.—The adult worms are found almost exclusively in the spiral valves of Elasmobranchs. They vary in size from 2 or 3 mm. to 30 or 40 cm. in length, but the majority measure about 3 or 4 cm. in length and 2 or 3 mm. in breadth. They never attain a size and grossness of such a parasite as *Dibothriocephalus latus*.

Larval forms.—The larval forms occur in a great variety of animals. They have been recorded from jellyfish, molluscs and bony fishes, but undoubtedly they will be discovered in other phyla of marine animals when investigations are extended. They are particularly abundant in bony fishes and occur in the largest numbers on the mesentery. One species of larva apparently shows a predilection for the lumbar muscles of its host, giving rise in this site to large abscesses. In the great majority of cases the larval form appears as a bladder resembling *Cysticercus cellulosae* or *C. bovis*. Through this transparent bladder, the larva, which

* A paper presented to Section D of the British Association, York, 6th Sept., 1932.

is merely the head of the adult worm, appears as a milky-white spot suspended within the cyst. The latter is surrounded by one or more secondary cyst walls, usually stout and fibrous, apparently secreted by the host, and often pigmented black or silver grey.

The larvae found in jellyfish and molluscs are, however, not usually bladder-like, but solid, and thus resemble plerocercoids; they are small, measuring 2 or 3 mm. only in diameter.

Pathogenicity.—The adult worms do not appear to be pathogenic. In my own experience the only pathogenic larva is *Tentacularia pillersi*, which gives rise to abscesses in the haemal arches of *Cossyphus axillaris*.

Amongst Elasmobranchs, the relation of the host to the parasite, and *vice versa*, is one of considerable importance. When the head of the parasite becomes attached in the spiral valve, its position is at first marked by a prominent hæmorrhagic patch. Later on, the hæmorrhage ceases and the position of the head of the worm is much less distinct. Calcification of the area then commences and results in the production of small calcareous nodules, with which the spiral valves of the old infected fish are studded. As a result of this calcification, the head of the parasite becomes subjected to pressure and breaks up, later on itself becoming calcified. The worm thus becomes detached from the wall of the intestine of the host, degenerates, and is passed to the exterior. Hooks of *Acanthobothrium ijimai* and *A. coronatum* have been found by the writer in the calcareous nodules which occur in the spiral valves of various species of *Trygon* and *Carcharias*, and there can be no doubt that the pathological reactions of the host have the effect of limiting the infestation.

Immunity.—Although careful work on this subject has never been attempted, it has been the writer's experience that, except in a few small species of *Trygon*, all Elasmobranchs in Ceylon and Indian seas are infected. This fact suggests continuity of infection and it would thus appear that little or no immunity is developed.

Eggs and gravid segments.—It is most unfortunate that we know nothing whatever about the eggs and gravid segments of the cestodes found in marine fishes. This circumstance arises from the fact that, in contradistinction to other cestodes, the mature segments of tapeworms from marine fishes are passed in the faeces of the host and apparently have a free existence in sea water, where the uterus develops, the

proglottides become gravid and the eggs ripe. A most important part of the investigations of future workers should be to examine the faeces of the fishes caught, with the object of extending our knowledge regarding gravid segments and eggs.

Life histories.—When one considers that, of the many thousands of tapeworms which have been recorded and described, the life history of only about a hundred species is known, it is not surprising that, with perhaps three or four exceptions, we know nothing relating to the life history of the cestodes of marine fishes. It appears abundantly probable, however, that the intermediate hosts (Medusae, Molluscs, Teleosts) become infected with the larvae through swallowing the eggs, and in some instances, perhaps, through devouring gravid segments. In the case of Teleosts at any rate, one presumes that the hooked embryo within the egg escapes into the lumen of the intestine, bores its way into a blood vessel of the mucosa or submucosa, and is then carried passively in the blood stream to its particular site. Here it leaves the blood stream and develops into the larval form. It is well known that bony fishes are devoured by sharks and rays ; the larval form thus swallowed apparently becomes adult in the spiral valve of these predatory and voracious fishes.

Herdman and Shipley and Hornell described larval Tetrarhynchids from the Ceylon pearl oyster which they believed were concerned in pearl formation in that animal. The adult worm *Tetrarhynchus unionifactor* was said to inhabit the spiral valve of *Rhinoptera javanica*. Unfortunately, the adult *T. unionifactor* has not been recorded since with certainty, and, in fact, it is doubtful whether it can be identified from the descriptions given. By far the commonest larval form in the Ceylon pearl oyster is a species of *Tylocephalum*, and, having regard to the frequency with which adult species of this genus are found in the marine Elasmobranchs of Ceylon, the writer is satisfied that the pearl-inducing larva is a species of larval *Tylocephalum*, the adult of which is not yet established. But as only six species of this genus have been recorded, the exact determination should not be surrounded with any particular difficulty.

Classification.—It is common knowledge that hardly any two authorities can agree on the classification of any group of animals, and I have learnt to be generous and not dogmatic on this subject as the matter is of very little importance. Speaking generally, the parasites found in marine fishes comprise a number of worms having a common anatomy which

varies within very narrow limits, and which have scoleces of one of the following types, viz.—(1) With four long, armed, protrusile proboscides (Tetrarhynchids); (2) With four lappet-like outgrowths from the head (bothridia), which may be subdivided or bear suckers, loculi or hooks (Phyllobothids); (3) Scolex composed of two parts, typically presenting a cottage loaf appearance; sometimes armed with suckers, or hooks, or both. The anterior part in one or two species, and the posterior part in one or two other species, may be divided into finger-like or feather-like processes (Lecanicephalids).

Economics.—With one exception, parasites of marine fishes have no economic importance. This arises from the fact that no one is concerned even if marine fishes suffer from toxaemia, leukocytosis, impaction of the gut, gid, cysticercosis, gastro-enteritis or other maladies, and the treatment of parasitic diseases in fishes has not yet been brought within the province of the veterinarian. Further, as far as is known, there are no parasites of marine fishes which are infective either to man or domesticated animals. There can, however, be little doubt that pearl formation in the Ceylon pearl oyster occurs round a species of larval tapeworm (*Tylocephalum*), the adult being found in certain sharks and rays which eat the oyster. Although hundreds of such larvae may be found throughout the tissues of a single oyster, it would appear that pearl formation only commences when such a larva for some reason or other dies, thus giving rise to a local irritation. This condition is followed by an active migration of certain cells in the tissues of the oyster which are normally concerned in secreting the pearly nacre of the shell. These cells surround the dead parasite and cover it with layer upon layer of pearly material so that a section of a pearl resembles a section of an onion.

Future Investigations.—In my opinion future investigations on the cestode fauna of marine fishes should be devoted in the first instance to the following three points, viz.—(1) Establishing definitely the identity of the pearl-inducing worm and the conditions relating to pearl formation round the larval parasite in the pearl oyster; (2) Collection and examination of specimens from new areas such as round the coast of Africa, the Arctic, South America, the Far East and Australia; (3) Examination of the faeces of sharks and rays caught in all areas, with the object of collecting information relating to the gravid segments and eggs of all species.

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On the Life-History of *Heterakis gallinæ*.

By Phyllis A. Clapham, B.Sc.

(*From the Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.*)

During the course of some experiments involving the Nematode worm *Heterakis gallinæ*, it became necessary to know the exact life history in order to interpret the results. On consulting the literature it became evident that the details of the life history had never been worked out accurately in either this worm or in any of its near relations. Furthermore, there was considerable controversy on the subject. For this reason therefore the whole morphology and life history were investigated in detail and some interesting points came to light.

The results recorded in this paper were obtained by the writer whilst working under a grant by The Medical Research Council to Professor Leiper, F.R.S.

Nomenclature.

Heterakis gallinæ was first described by Gmelin in 1790 : he, however, associated it with the genus *Ascaris*, which at that time contained a heterogenous collection of worms of all descriptions. The following year Frœlich re-described it, and though at first he uses the name of *Ascaris*, later he introduces the term of *Heterakis*.

In 1845 Dujardin used the new generic name for it and added three other species. He drew attention to the necessity for the new genus on the grounds of the inequality of the spicules and their dissimilarity, the position of the vulva and the nature of the uterus. He took the chicken Nematode (*H. vesicularis*, as he knows it) as the type species and noted the points of resemblance to the genus *Ascaridia*, already a well known genus—the mode of division of the uterus and the male tail being striking points of resemblance. *Ascaris dispar* was well known and he realised that this should be incorporated in the newly founded genus of *Heterakis*. He also took two parasites of batrachians—" *Ascaris brevicaudata* " and " *A. acuminata* " and added them to his genus *Heterakis*, though he was

careful to point out that there were certain fundamental differences in the genitalia, etc., which would probably bring about the creation of another genus for them.

In 1800 Zeder introduced his genus *Fusaria*, to which he allocated many varieties of worms, *Heterakis gallinæ* being one. This genus, however obtained very little hold upon the zoologists of the day, and from that time onward the genus *Ascaris* was used to accommodate this worm until Railliet once again used the name of *Heterakis* (1885), but the specific designation did not become fixed for very many years.

Gmelin was responsible for the term *gallinæ*, but this did not pass into the literature until recently, though he gave an easily recognisable description of the species. In 1791, the first year after its description, Frœlich mentions it, but gives it the name of *vesicularis*, and this was the name that was used for years, though other names were given and were used for varying lengths of time. Dujardin used this name, as also did Rudolphi in his "*Entozoorum Synopsis*," in 1819. He mentioned it twice and gave an accurate description of it, and also mentioned 11 hosts for it. The nomenclature of these hosts is not always accurate, but they can always be easily recognised as common game and domestic birds.

Railliet, in 1885, used the specific name of *papillosa*, confusing it partly with the worm *A. papillosa* (Bloch, 1782) obtained from the bustard. Neither the description of Railliet nor of Bloch dealt entirely with *Heterakis gallinæ*.

From that time onward, the worm was generally known as *Heterakis vesicularis* (Frœlich, 1791), and it was not until 1923 that Freeborn re-introduced the true name which now is known to be *Heterakis gallinæ* (Gmelin, 1790) Freeborn, 1923.

Distribution.

Heterakis is a small worm occurring in the cæca of domesticated birds. The species *gallinæ* is cosmopolitan and is particularly abundant in the British Isles. It lives in among the partly digested contents, mainly in the distal ends of the cæca, but it is not confined to that region, but has been found along the whole length and also in the small intestine and in the colon. They are parasites of birds, particularly game and domestic birds, and Cram (1927) has evolved a host list containing 22 genera and 37 species, some of which are, however, synonyms.

Infestation with *Heterakis* is very common, and of cæca obtained from a poultry dealer, 75·2 per cent. were found infested. Usually both cæca were involved, but occasionally one was free. The number of parasites varied from a single specimen to nearly 700. It is interesting to note in the latter case that the cæca appeared quite normal, in spite of such a heavy infestation.

Most writers record a fairly heavy percentage of infested fowls. Dujardin (1845) estimates 56·8 per cent., Ackert (1927), in Kansas, gives the percentage as 74·1, while Riley and James (1921), in two separate counts taken in Minnesota, record percentages of 78·1 and 62·9. That the distribution may be patchy, however, is shown in a paper by Roth, who, working at Breslau, found only 5·65 per cent. infested—*i.e.* 13 out of 230 chicks.

Similarly all writers seem to find a wide variation in the intensity of infestation. Most commonly less than 100 worms are present, but much heavier numbers have been recorded frequently, and even in the heaviest infestation the lumen is not occluded.

The recorded hosts for *H. gallinæ* are given here. The list comprises representatives from 20 genera and contains 33 species. It is modified from Cram (1927) and the synonyms given in brackets are the names used by her.

(1) CORVIDÆ. *Corvus cajanus.*
(2) ANATIDÆ. *Anas boschas* (Wild Duck).
A. tadorna.
Anser anser (*A. cinereus domesticus*) (Grey Lag Goose).
Chenopis atrata (Black Swan).
Tadorna tadorna (Common Duck).
(3) OTIDÆ. *Otis tarda* (Great Bustard).
O. tetrax (Little Bustard).
(4) TETRAONIDÆ. *Bonasa sylvestris.*
Lagopus mutus.
L. scoticus.
Tetrao bonasia.
T. lagopus.
T. urogallus (Capercaillie).
Tympanuchus (*Cupidonia*) *cupido* (Grouse).

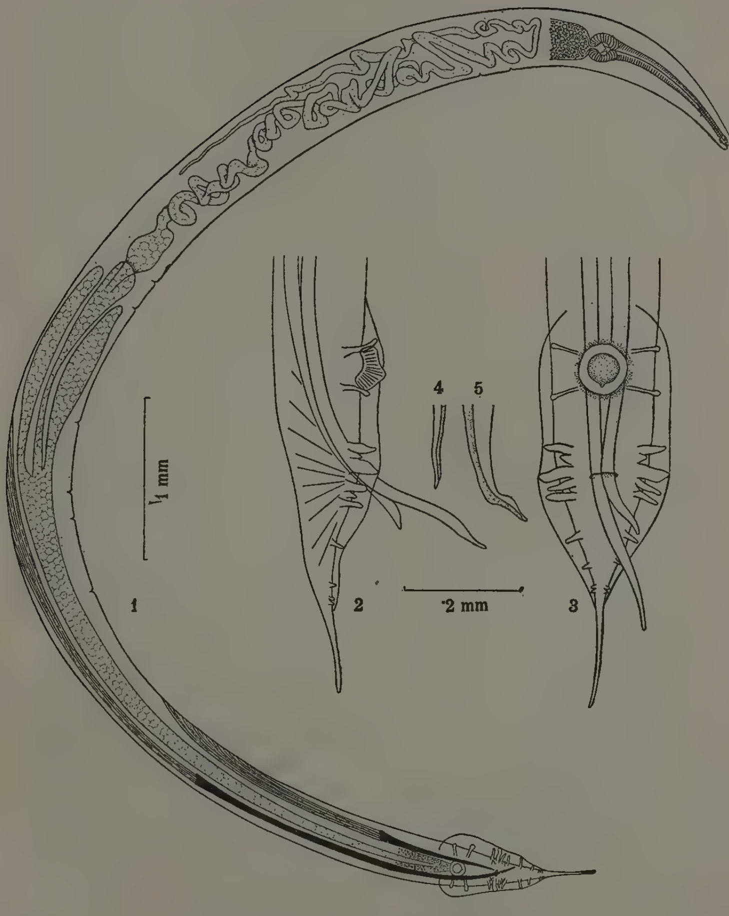
(5) PHASIANIDÆ. *Crysolophus pictus.*
Colinus (*Ortyx*) *virginianus* (Virginian Partridge).
Gallus gallus (Common Fowl).
Meleagris gallopavo (Turkey).
Numida galeata (*meleagris*) (Grey-breasted Guinea Fowl).
Pavo cristatus (Peafowl).
Perdicula (*Coturnix*) *communis* (Quail).
P. dactylisonans.
Perdix cinera (*perdix*) (Partridge).
P. coturnix.
P. saxatilis.
Phasianus colchicus (Pheasant).
Ph. gallus.
Ph. nychtemerous.
Ph. pictus.
Ph. veneratus.
Ph. versicolor.
Tragopan (*Ceriornis*) *satyra.*

MORPHOLOGY.

Heterakis gallinæ appears as a small whitish worm, rather opaque and with the anterior end usually flexed dorsally from the region of the œsophagus—the rest of the body lying straight.

It is covered with cuticle traversed by fine transverse striations about 3μ apart: the cuticle is expanded laterally into two flanges extending from shortly behind the mouth nearly to the end of the body. This cuticle is smooth.

Digestive System.—The mouth is terminal and is surrounded by three regular lips, one dorsal and two sub-ventral. The cuticle is somewhat thickened on the radial margins of the lips. Each lip is provided with two papillæ. The mouth aperture is triangular: round the aperture the cuticle is thickened. The mouth leads directly into a pharynx with thick walls. This passes back into the œsophagus which is a strongly muscular tube, expanded distally into a bulb. The total length in a full grown specimen is about 1 mm. The bulb is muscular and is provided



Morphology of *Heterakis gallinæ*.—Male.

Fig. 1. Adult male, showing the genitalia.
Fig. 2. Tail, lateral view.
Fig. 3. Tail, ventral view.
Fig. 4. Tip of left (short) spicule.
Fig. 5. Tip of right (long) spicule.

with a cuticular masticatory apparatus consisting of three well-marked dentate valves lying alternate with the lips—that is in the interlabial position. The neck of the œsophagus is provided with three fine rows of hinged rods, placed transversely. By these means, an extraordinarily strong suctorial action is effected. The intestine and rectum are normal.

Nervous System.—This consists of a slender ring loosely surrounding the neck of the œsophagus and placed about half-way along its length.

Excretory System.—The excretory pore lies in the mid-ventral line slightly behind the level of the nerve ring. It leads into a vesicle lying obliquely backwards. This receives the longitudinal ducts from the anterior and posterior parts of the body.

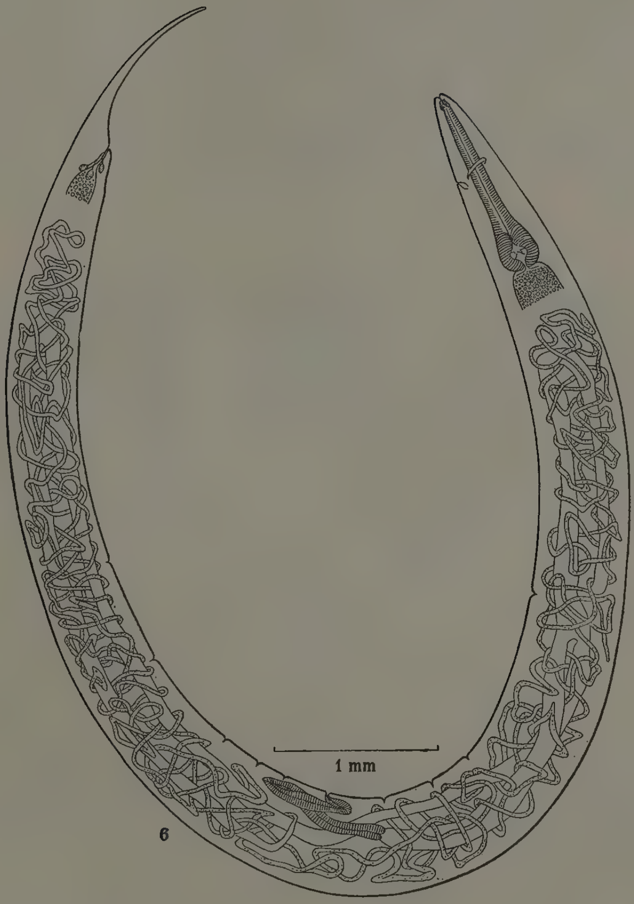
Male Genitalia.—The adult male varies in length from 7—12 mms. (Cram reports the figures as being a little larger.) The genital tube is single and begins as a fine rod of cells rather in front of the middle of the body. This rod passes forward, at first straight, but it gradually thickens and assumes a convoluted appearance. A short distance behind the œsophageal bulb it crosses the body and passes posteriorly, showing more convolutions. At about the level of the testis, the rod of cells becomes hollow and definite walls develop. This duct passes back into a seminal vesicle which throws off anteriorly three well-marked diverticula. The main stem runs down the body and opens into the intestine, just above the rectum. The whole apparatus is charged with sperms.

The spicules in *Heterakis* are typical—unequal and dissimilar : the right being about 2 mms. long, circular in cross section with a single flange and a blunt point, while the left averages about 0·65 mm. long, and is broad and flat with two lateral flanges : the tip is sharply curved and pointed. They lie in spicular sheaths and are provided with long retractor muscles. There is no accessory piece.

The wall of the rectum is provided with a large number of small unicellular glands.

The tail is straight, terminating in a blunt point : the cuticle being expanded into a well marked bursa supported by 12 pairs of papillæ, and with a chitinous pre-anal sucker. This is about 70μ in diameter and posteriorly the inner limit is marked by a small incision.

The papillæ are arranged as follows : Two pairs of pedunculate papillæ are associated with the sucker. These are followed by a group of six pairs in the vicinity of the ano-genital opening. Of these numbers four



Morphology of *Heterakis gallinæ*—Female.

Fig. 6. Adult female, showing the genitalia.

and seven are sessile, while numbers three, five, six and eight are pedunculate. They are thick and fleshy, particularly number five. A short way behind the ano-genital opening is a single pair of pedunculate papillæ while the last three pairs are sessile. Of these number 10 stands alone, and the last two are close together. Some variation occurs here: in some individuals only one pair occurs. That this difference is spontaneous and has no specific significance was shown in some chickens experimentally infested with eggs from a single specimen of *Heterakis*. In the resulting infestation both types of male were found—most with a double pair, but some had only a single pair.

Female Genitalia.—The length of the female varies from 8-16 mms. She is usually a bulkier worm than the male and the anterior end is more frequently flexed than the male. The genital tubes are double. The distal ends, reaching to the middle of the body, are compact straight rods running dorsally in opposite directions. Later they become convoluted and acquire a duct, thus becoming oviducts. They pass nearly to the levels of the rectum and œsophagus, when they cross to the ventral side of the body and pursue a very convoluted course towards the middle. They finally acquire a thin definite wall of flat squamous cells, enclosing a wide, dilated, sac-like cavity. These uteri pass forwards and backwards, turn again to the middle and finally unite somewhere between the vulva and the œsophagus, forming a common uterus. This passes backwards and becomes the vagina, which has thick muscular walls and encloses a narrow cavity. It is bent on itself roughly in the form of the latter S, and opens in the mid-ventral line shortly behind the middle of the body—the position of the vulva averaging about 52 per cent. of the total body length. There is no special ejaculatory apparatus, but the walls contain very powerful intrinsic muscles which must exert a strong action on the eggs as they pass along. There is a sphincter muscle at the junction of the common uterus and the vagina.

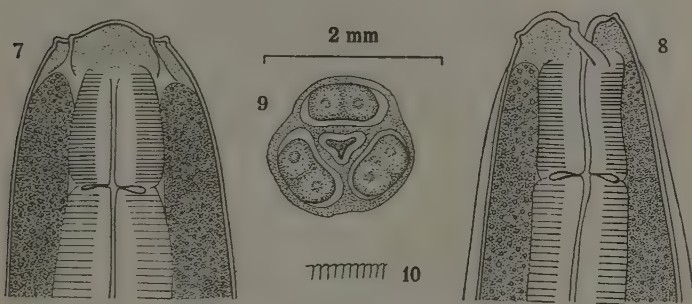
The tail of the female is long, about 1 mm. in length in a full grown specimen, and tapering, terminating in a blunt point. There are three large unicellular glands lying at the base of the intestine and opening into the rectum, close to the anus.

The eggs will be considered in the next section.

Life History.

Some difficulty has been met with in the past in elucidating the life history: the general opinion has been that the development inside the host is direct, taking place in the lumen of the cæca, though earlier writers have suggested that certain complications occur.

Galli-Valerio (1896) and Létulle and Marotel (1904) suggest that the larvæ may form cysts in the cæcal wall, though the latter writers suggest also that this is an abnormal occurrence, brought on possibly by reason



Morphology of *Heterakis gallinæ*.—Female.

Fig. 7. Dorsal view of the Anterior end.
Fig. 8. Lateral view of the Anterior end.
Fig. 9. Anterior view showing the arrangement of lips and papillæ.
Fig. 10. Skin Striations.

of an inadequate food supply or for increased protection. It is hardly to be considered that they would mature there. Graybill (1921) found the larvæ in the mucosa, and Uribe (1922) reports the occurrence of larvæ in the cæcal glands. The Oklahama Experiment Station (*fide* Cram) reports the death of a large number of chicks from pneumonia 8-10 days after feeding with eggs of *Heterakis gallinæ*, thereby implying that a migration, comparable with that of *Ascaris* takes place.

Other species, some of which are closely related to *H. gallinæ*, are not normally inhabitants of the lumen of the cæca. *H. isolonche* frequently forms tumours in the cæcal wall of pheasants, while Schwartz (1925) reports the occurrence of nodules caused by *H. beramporia* in chickens

in the Philippines. Itagaki (1930) comments on the development of "*H. vesicularis*": from experimental results he drew the conclusion that the larvæ hatch in the proventriculus in 36 hours and begin to penetrate the cæca on the fourth day after infestation, though some still remain in the intestine after 22 days. Some of his larvæ penetrated the cæcal glands, causing hypertrophy, and matured there. He examined poultry from various poultry yards and found 80 (percentage not mentioned) in which the cæca showed definite nodules in the subserous and muscular coats. These nodules contained Heterakid larvæ in various stages of development and several mature worms which could be identified as "*H. vesicularis*." He concluded that a migration had taken place early into the subserous and muscular coats and that the larvæ had matured there.

Mönnig (1926), when considering the life history of two species of *Trichostrongylus* in South Africa, and Fülleborn (1927) in considering *Uncinaria stenocephala*, note that the larvæ enters the mucosa of the stomach and intestine and refer to a similar phenomenon in *H. gallinæ* and other Nematodes. Fülleborn interprets it, with Mönnig, "as a remnant of a former migratory process."

The present writer succeeded in tracing the complete life history. The eggs are ovoid structures, bluntly rounded at both poles, and measuring from 70μ—80μ by 38μ—44μ, with an average of 73μ by 42μ. They are provided with a thick hyaline shell about 5μ in thickness and an inner somewhat irregular membrane. This is pierced by a clear lenticular space at one pole, sometimes slightly to one side, which may be of use in hatching. The thick shell is secreted in the terminal portions of the uterus, eggs in the more distant portions being without it.

No segmentation occurs in the cæca of the host, probably owing to lack of oxygen: they pass out in the single celled stage, such as occurs in the uterus of the female. Under favourable conditions of temperature, moisture and oxygen requirements, development to the infective eggs stage occurs in about 14—17 days. A fairly wide range of temperature is tolerated: development takes place at any termperature from 20° C. to 30° C., with an optimum of about 26° C. They can easily be cultivated in small Petri dishes, especially if they are well aerated. They develop in various dilute solutions of acids and disinfectants. For practical

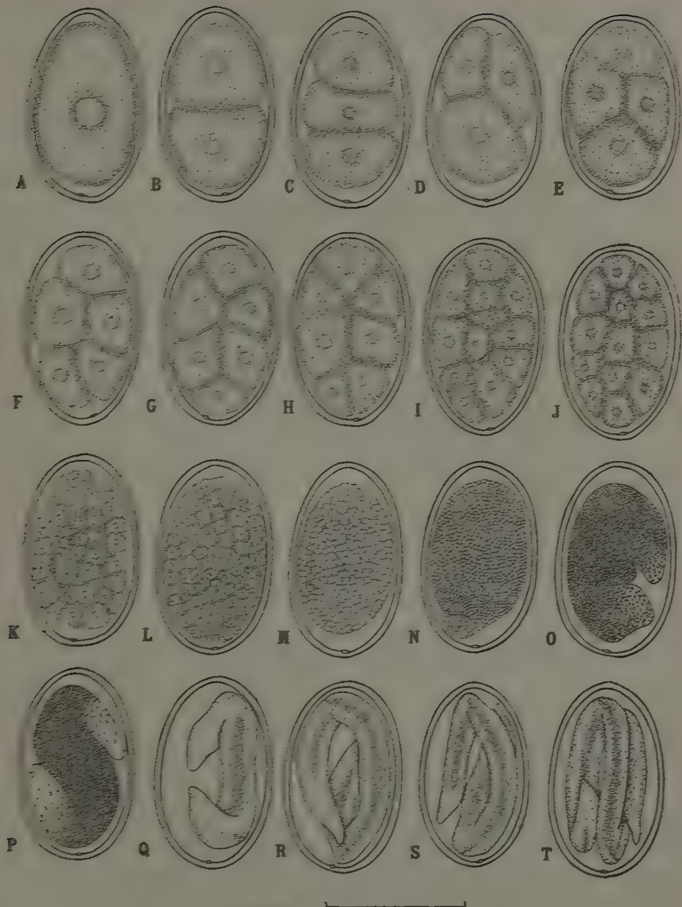


Fig. 11 ·05 mm

Development of the Egg of *Heterakis gallinæ*—Outside the Body.

Fig. 11. A.—Fertile egg as found in the fæces. B-H.—Segmentation Stages. I-M.—Morula Stages. N.—Early differentiation stage. O-Q.—Tadpole Stages. R-S.—Vermiform Embryos. T.—Infective Larva.

purposes the writer used 1 per cent. formalin, which kept down bacterial growths and in no way impeded hatching afterwards. Eggs kept in such a solution for seven months have been fed to a chick and have produced an infestation.

Segmentation occurs rapidly under such conditions. The first division occurs within 24 hours, and is quickly followed by further divisions, forming a morula in about 2-3 days. The cells continue to divide into a large number of small blastomeres which tend to be smaller at one pole than at the other. At about 6 or 7 days a depression forms between these two types of cells : this deepens and the embryo grows terminally, thus taking on a distinctly vermiform appearance. It continues to grow and becomes infective in 5 or 6 more days.

First Stage Embryo.—This occurs in the egg shell, and is of very short duration. It is only very slightly motile. The œsophagus has a distinct bulb terminally and a swelling on the neck, thus making it definitely double bulbed, and bringing it into line with the typical Nematode first stage larvæ. This moults, though it does not shed its sheath until it has been ingested by the host.

Second Stage Embryo.—The infective egg contains the second stage embryo, and it is seen coiled usually two or three times within the shell. The embryo rarely shows much activity, though slight movements do occur and can be induced by such local stimuli as change of temperature, etc. When removed from the shell by slight pressure, they usually give one convulsive contraction, thereby stretching themselves into an arc and then lie fairly still, except for slight rotation of the head and tail.

The embryo thus removed is seen to be about 235μ long by about 20μ broad. The skin is smooth and the tail tapers to a point in all specimens. The mouth is a simple pore, no lips being distinguishable, and leads directly into the œsophagus. This is elongated and filariform. Posteriorly it broadens slightly, but it cannot be considered as being bulbed. The cells which compose the " bulb " are large and glandular and contain a prominent nucleus. This passes into the intestine, the cells of which are large and spindle-shaped and contain a prominent nucleus. The rectum is slit like and opens ventrally.

The nerve ring encircles the œsophagus about half-way down : the excretory pore is at the same level. The excretory vesicle is not formed at this stage—the pore leading to a simple duct.

The genital primordium appears as a linear row of four cells, lying ventral to the gut : the protoplasm is coarsely granular and the nuclei large.

The measurements are : *Length*, 235μ ; *Breadth*, 20μ ; *Œsophagus*, 74μ ; *Tail*, 30μ.

Such infective eggs fed to a suitable host are capable of producing an infestation. A large number of them are carried mechanically through the gut and pass out unhatched. The others hatch after 1-2 hours in the first quarter of the intestine. During the hatching process the egg shell splits longitudinally from the micropyle already noted. The embryos pass slowly backwards and can be found in the cæca after 24 hours, when they differ in no respect save that of length from those removed from the egg shell.

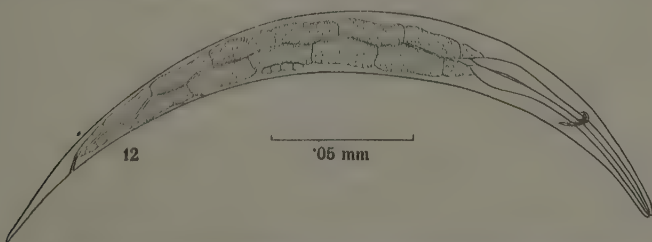


Fig. 12. Infective Larva of *Heterakis gallinæ* as found newly hatched in the small intestine.

The larvæ continues to grow and the genital anlage develops steadily and after 96 hours certain changes can be seen. There is a marked increase in length and structural alterations are taking place. Beneath the skin, the larvæ have defined lips and the skin is definitely striated. The genital anlage consists of a solid rod of cells extending for some distance close to the intestine. The œsophagus is relatively shorter and is beginning to conform to the adult proportions. It is still glandular and filariform.

The measurements at this stage are : *Length*, 640μ ; *Breadth*, 45μ ; *Œsophagus*, 196μ ; *Tail*, 120μ

The two sexes have, however, to be differentiated yet, and though the final differentiation does not take place until after the next moult, yet

at this stage some larvæ have a group of cells in the neighbourhood of the tail—the anlage of the spicules—and the tail is itself definitely shorter. These are destined to become males. Others have a group of cells near to the middle of the body forming the vulva. In this group also the rudiment is somewhat shorter and does not extend so far backwards, and the tail is more elongated. These will become females. A moult occurs at about 96 hours.

Fourth Stage Larvæ.—Development now continues at a slower pace. The last moult does not occur until the tenth day after infestation, and by this time considerable growth in length had taken place. A certain amount of variation also takes place now. Up to this time the larvæ have all conformed to a more or less standard length, the variation being negligible, but now they measure anything from 3-4 mms. in length.

The sexes can be readily distinguished after eight days—the tail is then very definitely different in the two sexes, and also they are taking on the general shape and configuration of the adults. The female is long and tapering. The male has grown very much less in length, but has become rather short and stumpy. Considerable activity is taking place in the neighbourhood of the rectum: as has already been mentioned, a group of cells—the anlage of the spicules—can be distinguished at about the fourth day after infestation. Five or six days later these have divided a fair number of times, forming a rod of small granular cells. Chitin now begins to be laid down and the spicules become differentiated. They do not become fully developed for another two days, but the beginnings are obvious at this stage.

At the same time, too, buds destined to form the papillæ are obvious in the neighbourhood of the ano-genital opening and smaller ones occur just posterior to these. Anteriorly a group of epidermal cells become active in the secretion of chitin and the beginnings of the pre-anal sucker are laid down. So far, however, there is no trace of bursa. The worm is, however, preparing for its final moult—the intestine is packed with fat globules and the larva is less active.

In the female the position of the vulva is laid down and the cuticle forms a small protuberance here.

In all specimens, however, activity is occurring in all parts of the worm. Considerable growth has taken place in the region of the intestine. The

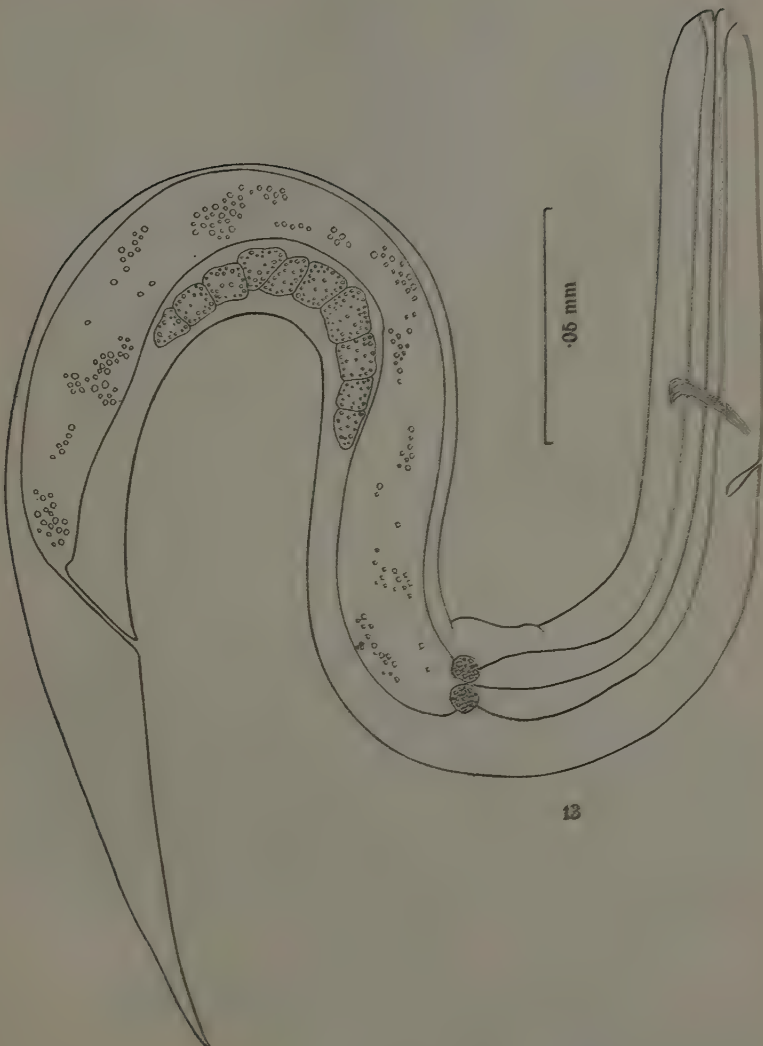


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Fig. 13. Third Stage Larva of *Heterakis gallinæ* found in the Cæcum.

cells of the œsophagus are changing from the glandular type to muscular. The worms are now ready to take on the adult form, after which only growth in length will take place. The last moult takes place at about the 10th day and young adults emerge.

To summarise the stages :—

(1) First stage is confined to the shell and has a double bulbed œsophagus. The first moult occurs in the shell.

(2) The infective embryo has a filariform œsophagus. The second moult occurs in the cæca of the host about 48 hours after infestation.

(3) The third larva has definite lips and a striated skin. The genital anlage develops in the direction of differentiation of the sexes and the position of the vulva and of the spicules can be seen. The third moult occurs after about 96 hours.

(4) Differentiation continues. Chitin is being laid down and the papillar buds are seen in the male. The fourth moult does not occur until about the tenth day.

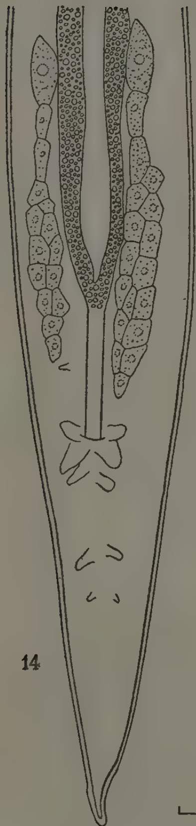
(5) Young adults emerge from this last moult, typical in all morphological respects for the species. Maturity is not reached for another 12 days, and eggs can be found in the fæces at about the twenty-fourth day after infestation.

Migration Within the Host.

As has already been mentioned, the possibility of a migration within the host has already been considered by earlier writers (Oklahama Experiment Station) and others, while not committing themselves to a definite statement, consider the larvæ to have a stage of parasitism within the tissues (Graybill, 1921 ; Mönnig, 1926, and others already mentioned). With a view to the investigation of these statements chicks were fed very large doses of *H. gallinæ* eggs—about 120 adult females being given to each bird. They were killed after 6, 12, 24 and 48 hours and the organs examined.

Fourth Stage—Larva of *Heterakis gallinæ* about to moult.

Fig. 14. Tail of male—ventral view.
Fig. 15. Tail of male—lateral view.
Fig. 16. Tail of female—lateral view.



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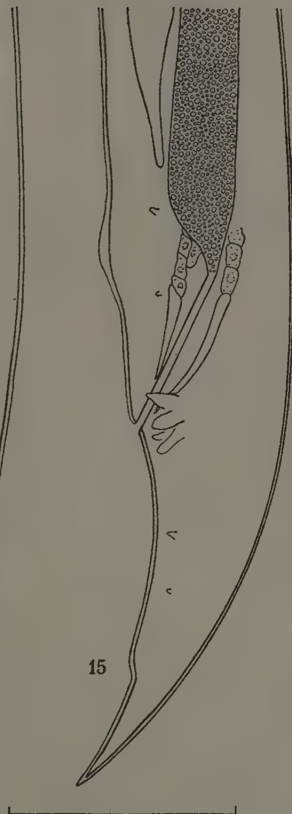

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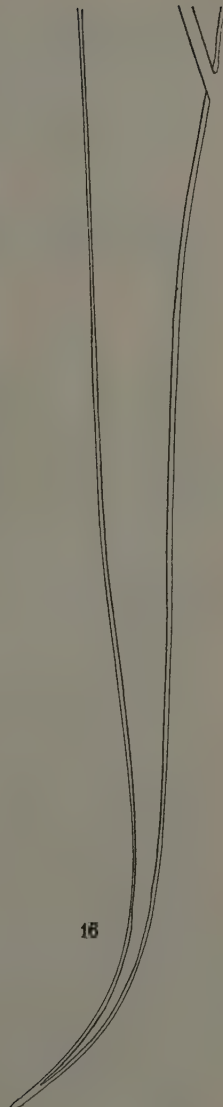

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(1) Killed after 6 hours. A few larvæ were found hatched in the first quarter of the intestine and a very few infertile eggs among them. Elsewhere no larvæ were found.

(2) Killed after 12 hours. Similar results as in case (1), except that the larvæ were further down the gut.

(3) Killed after 24 hours. Larvæ were found in the last half of the intestine, in the large intestine and in the mouth of the cæca. While full counts of larvæ were not made, there was a definite collection of larvæ in the region of the mouth of the cæca. None were found in the body organs.

(4) Killed after 48 hours. The larvæ were all found in the lumen of the cæca.

In each case a complete examination was made of the gut, blood stream liver, heart, lungs, trachea and peritoneal cavity. The organs were removed into physiological saline and the cœlom washed out with the same substance. Blood films were made from heart blood (left auricle), subclavian vein, portal vein and from a liver smear. The liver and lungs were examined partly by means of Bærmann extractions in saline and partly by direct examination of teased up portions. The trachea was examined by scrapings.

At no period was a larva found in any organ other than the gut and hence we may reasonably assume that no migration takes place.

Next in order to investigate Mönnig's statement that the larvæ of *Heterakis* pass into the mucosa and later return to the lumen of the gut, chicks were examined 48, 72, 96 and 120 hours after infestation. The cæca and terminal portions of the small intestine were alone examined. The contents were washed into saline and the mucosa observed under a low magnification. A healthy appearance was always seen, but scrapings were always made and examined, but no larvæ were ever seen. Likewise with sections—many were cut from each cæcum examined, but no larvæ were ever seen in close proximity to the wall of the cæcum. On one occasion the cæca showed two small hæmorrhages, but on further examination this was shown to be a congested capillary, and the walls of the vessels were quite unbroken and no larvæ were seen in association with this.

Summing up the evidence therefore we may say that the life history

of *H. gallinæ* is direct and involves no intermediate host. A period of development outside the host is necessary while the eggs become infective. After being ingested by the host the larvæ hatch, pass direct to the cæcum and mature there in the lumen.

Acknowledgments.

The writer would like to express her indebtedness to the Staff of the Imperial Bureau of Agricultural Parasitology for much help in obtaining the early literature dealing with the subject.

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On a New Trematode of the Genus *Astiotrema* Looss, 1900, from the Intestine of a Tortoise, *Chitra indica.*

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Looss (1898) described a trematode, *Distomum reniferum*, from the intestine of a turtle, *Trionyx nilotica*. Later, while reviewing the genus *Distomum* of Retzius, 1782, the same author (1899) described a new form, *Astia impleta*, from the intestine of *Tetrodon fahaka* and also transferred his former species, *Distomum reniferum* as a type species of this new genus *Astia*. The generic name was subsequently changed to *Astiotrema* Looss, 1900 and both the species described originally under *Astia* are now included under this new name. Odhner (1911) redescribed both the species and added considerably to our account of these forms. Other species have since been added to the genus *Astiotrema*. The latest addition to our knowledge of the genus *Astiotrema* is that by Mehra (1931) who has given an interesting account of two new species from the tortoises of Allahabad, and has, besides, given a very useful table and key for the identification of the various species of the genus under review. The present paper deals with an account of another new species of the genus obtained at Lucknow, from the intestine of a tortoise, *Chitra indica*, from the river Gomti.

Astiotrema indica n.sp.

A large number of specimens of this trematode were collected from the intestine of the tortoise, *Chitra indica*, in 1929. Some of these worms were flattened by pressure of coverslip, stained and mounted, while others

were fixed in corrosive sublimate for sectioning. It may be remarked here that the specimens were fairly thick and opaque.

The body of the trematode is elongated, flattened dorso-ventrally, and is thick walled. The specimens flattened by a pressure of cover-glass measure 10 mm. to 11 mm. in length, with a maximum breadth of 2·15 mm. to 2·3 mm., in the region between the two testes. The body is rounded at either end and is covered over with minute spines, 20μ long. The spines are more numerous in the anterior part of the body in front of the acetabulum. They are sparse in the posterior half of the body. They are not shown in the diagram, owing to their minute size.

The oral sucker is circular and is terminal in position. It measures 0·38 mm. to 0·4 mm. in diameter. The ventral sucker is also circular in outline and is almost equal to the oral sucker in size, being 0·4 mm. in diameter. It is situated at about 1/5th of the body length from the anterior end. The genital pore is situated at 0·3 mm. in front of the ventral sucker, about midway between the centre of the acetabulum and the point of bifurcation of the intestine. The mouth lies in the centre of the oral sucker and leads into a short prepharynx, 0·2 mm. long. The pharynx is pear-shaped, measuring 0·25 mm. by 0·32 mm. in dimensions, and has thick muscular wall. The œsophagus is very long, 0·9 mm. to 0·98 mm. in length and its lumen is rather wide. It divides into two intestinal cæca in front of the ventral sucker. The bifurcation of the intestine is more near the ventral than the oral sucker. The cæca run to the posterior end of the body and possess slightly lobulated outer margin. This is particularly noticeable at the anterior end near the intestinal bifurcation.

The excretory pore is subterminal and ventral, 0·16 mm. in front of the posterior end of the body. The excretory bladder is long with slightly sigmoid course and extends for about half the body length as far forwards as the anterior boundary of the anterior testes. Here it divides into two short cornua, one on either side. Each cornua, after a short course, divides into an anterior and a posterior branch, bearing groups of flame cells.

The ovary is sub-spherical and notched at the posterior end. It is broader than long and measures 0·44 mm. by 0·35 mm. It lies on the left side of the body, the sinistral position of the ovary is rather unique

in the genus *Astiotrema*. From the notched posterior end of the ovary arises a narrow oviduct, which is joined by the duct of the receptaculum seminis and the Laurer's canal. Slightly to the left of the union of these ducts, it receives also the common vitelline duct and forms the oötype. Here at the oötype the duct is surrounded by a large number of unicellular shell glands. This point, the oötype, is situated in the median line between the ovary and the receptaculum seminis.

The receptaculum seminis is very large and prominent, a feature in which the genus *Astiotrema* is peculiar from all other genera of the Lepodermatidæ. Here in this genus the receptaculum seminis is much larger than the ovary, being 1·25 mm. long by 0·4 mm. to 0·5 mm. broad. It is semilunar in shape and is placed transversely to the long axis of the body behind the ovary. The duct of the receptacle arises from its left side.

The vitelline glands are extensive structures, laterally situated on either side of the body, mainly occupying a space outside the cæca, but partly overlapping them, both dorsally and ventrally. They extend from the acetabulum to the anterior end of the posterior testis and consist of pear-shaped or oval follicles arranged in groups. The lateral vitelline ducts, anterior and posterior, lead into a transverse duct in front of the receptaculum seminis and these unite each other between the ovary and the receptacle to form a median duct joining at the oötype.

The uterus arises from the oötype on the side opposite to that from which it receives the oviduct. It then coils backward into a descending limb to the posterior end of the body and then extends forward into an ascending limb. The various uterine coils run between the intestinal cæca and during their upward and downward passage they pass between the two testes, as is the condition in the members of the family Lepodermatidæ. The terminal part of the uterus, in front of the ovary, is muscular and is termed the metraterm. It is situated on the left side of the seminal vesicle and the cirrus, but on approaching the acetabulum it turns over to the right side of that organ before opening at the genital pore in front.

The eggs are oval and thick-shelled and measure 38μ by 14μ in dimensions.

The testes lie in the posterior half of the body behind the ovary and

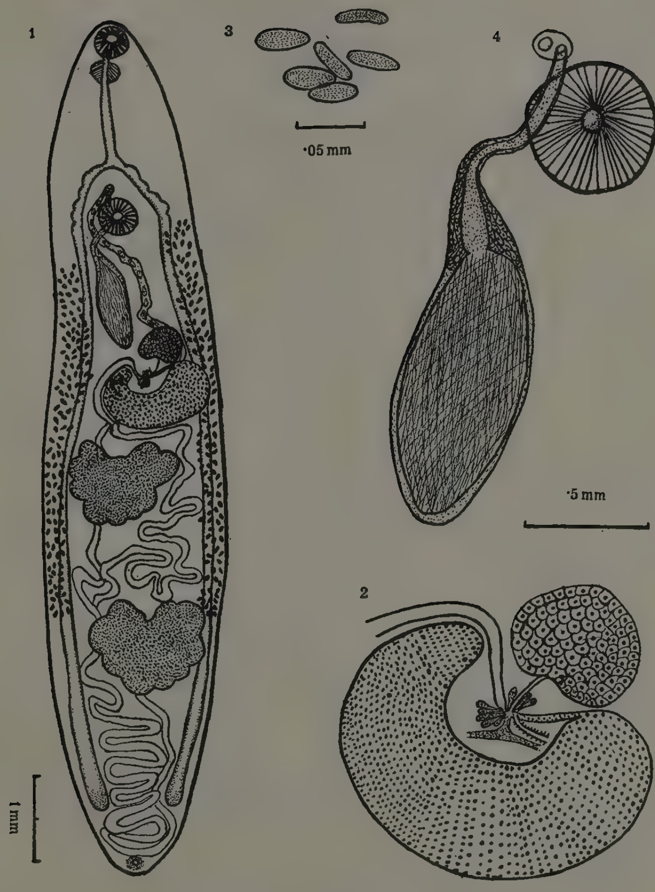
are large massive organs situated diagonally one behind the other. The posterior testis is larger than the anterior and is divided into nine to ten lobes. It is broader than long and measures 1·4 mm. by 0·8 mm. The anterior testis is also lobed like the posterior testis, having nine to ten lobes, but it is smaller in size, being 1·3 mm. by 0·75 mm. It is also broader than long and is separated from the posterior testis by the ascending and descending uterine coils. It is 1·3 mm. in front of the posterior testis.

The cirrus sac is a large oval bag, extending far behind the acetabulum, reaching the anterior end of the ovary. It is of a characteristic shape and is broad oval in its basal part and narrow in its terminal part. In its basal part it encloses a large pear-shaped seminal vesicle, 1·2 mm. long, running parallel to the long axis of the body. It fills up the greater part of the vesicular end of the cirrus sac. The terminal part of the cirrus sac bears a broad cirrus produced into a long narrow tube. The cirrus at its base is surrounded by a large number of unicellular prostate glands, enclosed within the cirrus sac. The cirrus makes a distinct coil in its course before opening at the genital pore in front of the acetabulum. (Fig. 4.)

The present species shows very close resemblance to *Astiotrema elongatum* in general characters. But it could be readily distinguished from it by the shape of the testes, shape of the ovary, the large semilunar receptaculum seminis transversely disposed across the body, the lobulations and the bifurcation of the intestinal cæca and the relative size of the suckers. The peculiarity of the cirrus in forming a definite coil in its course and the transverse disposition of the receptaculum seminis are points in which it is unique amongst the various species of the genus *Astiotrema* and these alone sufficiently justify the creation of a new species for these trematodes from *Chitra indica*.

Discussion.

Baer (1924) erected a new sub-family, Astiotreminæ, for the reception of the two genera, *Astiotrema* and *Glypthelmins*, on the character of the reduced cirrus pouch and the presence of the receptaculum seminis. The cirrus is not reduced, at any rate in the genus *Astiotrema*, and this is



EXPLANATION OF FIGURES.

Fig. 1.—*Astiotrema indica* n.sp., ventral view.

Fig. 2.—View of Female sexual organs, showing the oötype and the connecting ducts. Semi-diagrammatic.

Fig. 3.—Eggs of *Astiotrema indica* n.sp.

Fig. 4.—Cirrus sac and contained organs of *Astiotrema indica* n.sp.

mentioned by Looss (1899), Odhner (1911) and is further confirmed by Mehra (1931) and by myself in the present communication. This led Mehra to discard the sub-family Astiotreminæ Baer, 1924 and to include the two genera, placed under it by Baer, into the sub-family Lepodermatinæ. This, however, does not appear to be desirable. The sub-family Lepodermatinæ has characters that differ remarkably from those of the two genera of *Astiotrema* and *Glypthelmins*. Thus, the cirrus pouch in the genus *Astiotrema* is narrow and fine in its terminal part and broad and sac-like in its basal part. In Lepodermatinæ it is semilunar in shape. This feature coupled with the presence of a large receptaculum seminis in *Astiotrema* and its ally appear to be sufficiently important to justify the retention of a separate sub-family for these two genera. The writer agrees with Mehra (1931) in the undesirability of multiplication of sub-families on minute differences, but in the case under review it is not so. The differences are fairly important and affect remarkably the affinities and systematic position of the genera in question. The complete absence of the receptaculum seminis in Lepodermatinæ and its presence in a well-developed form in *Astiotrema* and *Glypthelmins*, as also the peculiarity of the cirrus pouch in the two groups of trematodes definitely separates them from each other and on these differences alone it does not appear reasonable to adhere to the view expressed by Mehra for fusing them with the Lepodermatinæ. Hence it is proposed to create a separate sub-family for the genera *Astiotrema* and *Glypthelmins*. This has already been done by Baer (1924) who erected Astiotreminæ to include them, though the diagnosis given by him is incorrect. It is a pity that a sub-family is to be retained on characters entirely different from those mentioned by its author, but under the existing rules of Zoological Nomenclature it is only possible to emend the definition of a previously occupied name. Hence we give the following emended diagnosis of the sub-family Astiotreminæ :—

" Lepodermatidæ, characterised by the presence of a large receptaculum seminis. The cirrus pouch broad and sac like in its basal part and narrow and fine in its terminal part. Genital pore in front of the acetabulum. Testes spherical or lobed. Excretory bladder long Y-shaped. Parasites of Amphibia and Reptiles."

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Journal of Helminthology, Vol. XI, *No. 2, March,* 1933, *pp.* 95-100.

On a New Species of *Enterobius* from the Marmoset *(Callithrix jacchus).*

By S. GLADSTONE SOLOMON, B.Sc., Ph.D.
(*Ministry of Agriculture Research Scholar, London School of Hygiene and Tropical Medicine.*)

THE material upon which this addition to the ever-growing genus *Enterobius* is based, was collected from the cæcum and large intestine of two marmosets which died at the London Zoological Gardens during the winter of 1931-32. The material came to me in the course of the routine examination of Helminthological material from the Prosectorium, and as the genus is one of importance in relation to human hygiene, this species seems to the writer to merit a brief description.

Recent additions to the genus have been made by Buckley (1931, p. 133) and Cameron (1929, p. 161). The latter adds two new species to the genus (*E. atelis* and *E. pitheci*). In an interesting list and summary, Cameron points out that each genus of primate has its peculiar species of *Enterobius*, suggesting that, as a result of the frequency of auto-infection, the parasite has evolved parallel to its hosts, but more slowly. Buckley, however, records two species of *Enterobius* from the same specific host (*E. lagothricis* and *E. duplicidens* from *Lagothrix humboldtii*). Each species of *Enterobius* would appear to show closest affinities with those other species which come from a host closely related to its own.

The host, in this case, is *Callithrix jacchus* (Linn., 1758), Erxl., 1777. Stiles and Hassall (p. 565) show that the generic name *Callithrix* Erxl., 1777, has priority over the much more frequently used *Hapale* Illiger, 1811. The locality is given only as South America. The host belongs to the family *Hapalidæ* which, with the *Cebidæ*, comprises all the New World (Platyrrhine) monkeys. There does not appear, so far, to be any species of *Enterobius* recorded from the genus *Callithrix*. (Stiles and Hassall, pp. 565-567).

Description of *Enterobius callithricis* n.sp.

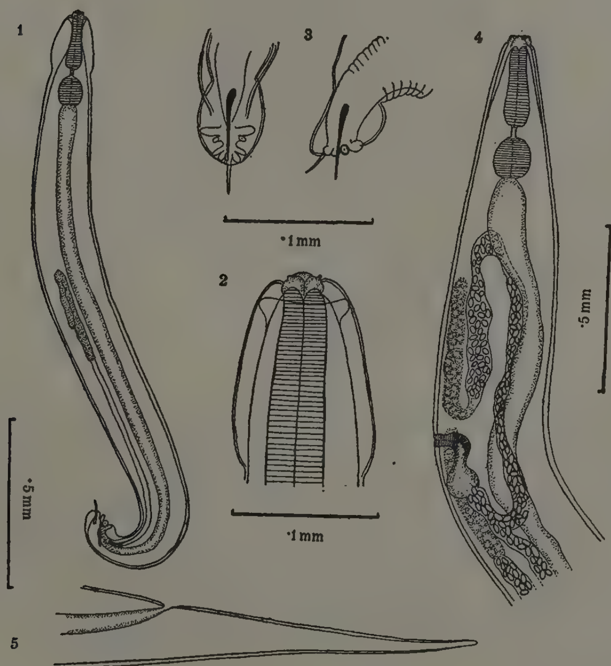
Description of Male.

The male specimens studied measured from 1·56 mm. to 1·89 mm. in length, the maximum breadth recorded being 0·149 mm. The cuticle shows fine annular striations which are lacking in the female. The head is surrounded by a cuticular swelling which varies in size and is much more developed in the male than in the female. There are three lips, two of which bear very tiny papillæ. Some male specimens showed an annular constriction just behind the head, forming a kind of " collar." The œsophagus measures 0·26 mm. to 0·31 mm. in length and therefore occupies about 1/6th of the total length. It is typically oxyuroid in shape with a very well marked constriction and an almost spherical second bulb. The tail is sharply recurved and terminates in a bristle measuring 16μ to 20μ in length. There are a dorsal and a ventral caudal cuticular swelling and four pairs of subterminal caudal papillæ of which the anterior pair is the largest. The single dagger-shaped spicule has a very consistent length of 44μ and terminates anteriorly in a knob. There is only a single set of male genitalia. In the specimen figured the slender testis gland extends forward to a point 0·66 mm. from the tail where it recurves for a distance of 0·12 mm. It is followed by a uniform genital duct which cannot be differentiated into vas deferens and seminal vesicle. The ano-genital opening is sub-terminal and is surrounded by the caudal papillæ. There are no buccal teeth in either sex.

Description of Female.

The average maximum length would appear to be about 5 mm. (though some are not quite so long) and the maximum diameter 0·33 mm. The lips are as in the male, with very minute papillæ, but the cephalic cuticular swelling is much less marked and may be absent. The diameter of the head varies from 108μ to 120μ. The œsophagus, similar to that of the male in shape, is only very slightly larger in size (0·41 mm. to 0·42 mm. long) and therefore occupies a much smaller proportion of the whole length (1/12th in the female : 1/6th in the male). The tail, which tapers gradually and ends in a blunt termination, measures 0·83 mm. to 0·91 mm. in length. The vulva divides the body into the proportion of 12 : 38, *i.e.*, is placed near the posterior end of the anterior quarter. The vagina begins as a short muscular tube running at right angles to the cuticle and

then bends sharply backwards and enlarges into a non-muscular portion into which the two uteri discharge their contents. There is a very short common uterine duct running backwards, and this divides into the two uteri. One recurves and runs forward to a point near the end of the œsophagus, whence it curves back to terminate, just in front of the vulva,



Enterobius callithricis n.sp.

Fig. 1.—Complete male specimen.
Fig. 2.—Head of male.
Fig. 3.—Tail of male : lateral and ventral view.
Fig. 4.—Anterior part of female showing reproductive organs.
Fig. 5.—Tail of female.

in the anterior ovary. The posterior ovary commences close to the vulva, and a little further back it joins the forward loop of the posterior uterus. The ova measure 28μ to 32μ by 60μ to 72μ.

Discussion.

So far as the writer can determine, this is the first species of *Enterobius* described from any of the marmosets (Hapalidæ), and it does not exactly correspond with any of the species described from other monkeys. Its closest affinities are with *Enterobius microon* (v. Linstow, 1907) from *Aotus trivirgatus* (= *Nyctipithecus trivirgatus*, the Douroucouli monkey), a Brazilian species which has certain morphological characteristics in common with the Marmosets (teeth, brain, non-prehensile tail). Our specimens show a general resemblance to this species, as described by von Linstow (1907, p. 266). But in *E. microon* the œsophagus is much longer, (occupying 1/2·3 of the total length in the male and 1/6th in the female). Also the male of *E. microon* has only one pair of caudal papillæ (as against four pairs in our specimens). The spicule is slenderer and the ova are smaller in *E. microon.* On the other hand the cephalic cuticular swellings, and cephalic groove (" collar ") are common to both species.

Enterobius minutus differs in its larger size and longer œsophagus: and *E. atelis* and *E. duplicidens* in the possession of buccal teeth. *E. trypanuris* may be distinguished by possessing five pairs of male caudal papillæ and a longer spicule: and *E. sceleratus* by its characteristic head. *E. lagothricis* has buccal teeth and the circumoral lips have a bi-laterally symmetrical form and it has a relatively longer œsophagus. These points appear sufficient to differentiate the species under discussion from any other species of *Enterobius* so far described from New World monkeys: and accordingly the name *Enterobius callithricis* n. sp., is proposed.

This new species furnishes further evidence for the theory that the genus *Enterobius* evolved with, and parallel to, its primate hosts; and that each genus of the Primates has its own specific *Enterobius*. If this is accepted, the possibility of human infection through pet marmosets can be disregarded.

A summary of the points of difference between the species of *Enterobius* from New World monkeys is now appended.

Species.	Host.	Length of ♂.	Length of ♀.	Length of ♂ spicule.	Length of ♂ tail (bristle).	Tail of ♀.	Oesophagus of ♂	Oesophagus of ♀	No. of caudal papillæ in ♂.	Relative position of vulva in ♀.	Buccal teeth.	Buccal lips.	Cephalic cuticular swellings.	Size of ova.
E. callithricis	Callithrix jacchus	1·76	5·0	44μ	16–20μ	0·87	$\frac{1}{6}$	$\frac{1}{12}$	4×2	12 : 38	—	3	♂+ less in ♀	60–72μ × 28–32μ
E. microon	Aotus trivirgatus ...	1·42	4·43	49μ	23μ	1·1	$\frac{3}{7}$	$\frac{1}{6}$	1×2	9 : 25	—	3	+	42μ × 23μ
E. minutus...	{ Alouatta seniculus Ateles paniscus ...	3·0	8·5	40–55μ	10–11μ	1·7	$\frac{1}{4}$	$\frac{1}{4\cdot4}$	3×2	Ant. $\frac{1}{3}$	—	3	+	41·8μ × 28·8μ
E. atelis ...	Ateles spp.... ...	1·5	6·0	42μ	25μ	—	$\frac{1}{6}-\frac{1}{7}$	$\frac{1}{6}-\frac{1}{7}$	4×2	2 : 3	+	1+ (2)	small	40μ × 20μ
E. lagothricis	Lagothrix humboldtii	1·56	5·0 – 6·0	44μ	15μ	—	$\frac{1}{4\cdot4}$	$\frac{1}{8}$	4×2	1 : 3·6	+	2	+	38–40μ × 22μ
E. duplicidens	Lagothrix humboldtii	1·6	4·6	52μ	none	—	$\frac{1}{4\cdot2}$	$\frac{1}{5}$	4×2	1 : 3·5	+	2	+	38–40μ × 22μ
E. sceleratus	Saimiri sciurea and S. örstedii ...	1·25	6·5	20–30μ	15μ	1·5	—	$\frac{1}{11}$	4×2	1 : 2	small	3	+	55μ × 27μ
E. trypanuris	Pithecia monachus	2·0	6·7	70μ	12μ	0·78	$\frac{1}{6\cdot6}$	$\frac{1}{7\cdot3}$	5×2	1 : 2	—	2	small	45μ × 30μ

Measurements in millimeters except where stated to be in microns.
Measurements of œsophagus given as fractions of the whole length.

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Journal of Helminthology, Vol. XI, *No.* 2, *March*, 1933, *pp.* 101-104.

A Note on a New Species of *Breinlia (FILARIIDÆ)*, from a Tree Kangaroo

By S. Gladstone Solomon, B.Sc., Ph.D.
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In June, 1931 a small collection of parasites from the Prosectorium of the Zoological Gardens of London was found to contain a number of long slender filaria worms from the peritoneal cavity of a tree Kangaroo. Upon examination these worms appeared to represent a new species of the genus *Breinlia* which was erected by Yorke and Maplestone in 1926 to contain *Filaria trichosuri* Breinl, 1911 (not 1913 as quoted by Yorke and Maplestone), from the abdominal cavity of the Australian opossum (*Trichosurus vulpecula* Kerr).

In this case the host is *Dendrolagus inustus* Schlegel and S. Müller, the grizzled grey tree kangaroo from New Guinea. It had lived for 7 months in the London Zoological Gardens.

The worms are long and filiform: the females more than twice as long as the males. The males measured gave a length of 8 cm. to 8·3 cm.; the females 16·2 cm. to 18·2 cm. The cuticle shows regular fine annular striations except at the posterior end.

There are either 2 or 3 pairs—probably 3—of insignificant perioral papillæ measuring from 8μ to 12μ in length. There is a very small spherical buccal capsule leading into a simple muscular œsophagus which has a length of 2·1 mm. to 2·6 mm., measuring 40μ to 60μ across at the anterior end and 80μ to 100μ at the posterior thickening. The nerve ganglion is 236μ to 260μ behind the head.

The Male.—The head is 64μ to 68μ in diameter. The greatest thickness is 280μ in the middle: the tail terminates in a single papilla. The buccal capsule (vestibule) is 8μ to 12μ deep. The cloaca is between 400μ and 500μ from the tail—its position is not absolutely constant—and is flanked by papilliform lips 12μ long. The two spicules are unequal in

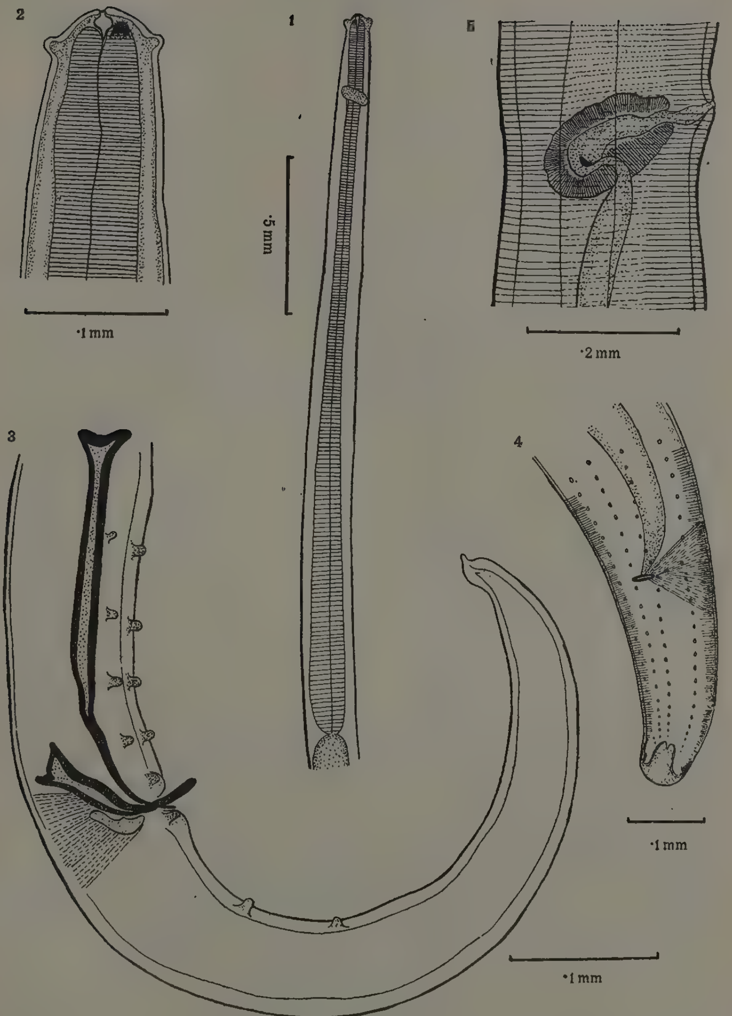
size. The long one, which consists of an irregular thick anterior portion and a slender filiform posterior portion is 240μ to 280μ long and has a greatest diameter of 24μ to 28μ, while the small spicule is short and stumpy and measures 80μ to 96μ long by 24μ to 28μ across.

There are 4 pairs of preanal and 2 pairs of postanal papillæ, besides the cloacal lips. The tail is twisted. A gubernaculum is present measuring 32μ to 36μ in length.

The Female is about twice as long as the male. The head measures 64μ to 76μ across: the œsophagus is 2·1 mm. to 2·6 mm. long and grades in thickness from 40μ to 48μ at the anterior end to 80μ to 100μ posteriorly. The greatest thickness of the body is 460μ to 480μ in the middle. In the œsophageal region it measures 120μ to 140μ across and in the posterior quarter 180μ to 200μ. The position of the anus in relation to the tail is variable and the vulva is also variable in position and is marked by a slight protrusion. Three specimens gave the distance from the vulva to the posterior end of the œsophagus as 2·32 cm., 2·5 cm. and 3·2 cm. The vulva leads into a short tubular vagina which runs in a posterior oblique direction and is enclosed by a muscular sheath. The cuticular striations are present in both sexes but the distances between each pair are greater in the female, the buccal capsule is deeper, measuring 15μ to 16μ. There are very minute pre- and postanal papillæ, which are very difficult to count, but probably number about 25 pairs on each side of the body, and the tail terminates in a single pair of large papillæ.

When compared with the description of *B. trichosuri*, the species under consideration shows differences as regards the following points:—(1) smaller size, (2) smaller spicules, (3) a different arrangement of caudal papillæ (4) the vulva is situated further back. All these points are well-marked. Furthermore, the œsophagus is here larger in proportion to the total length and the anus of the female and cloaca of the male are placed further back. Also the lips flanking the male anogenital opening are missing in *B. trichosuri*, and the genitalia extend further in the posterior direction in the present species. The minute caudal papillæ of the female do not appear to be present in *B. trichosuri* but they may easily have been overlooked as they are very insignificant. Embryos were squeezed out of the uterus and were found to measure 280μ to 300μ long and 8μ thick in the middle. They have no sheath.

The chief points of difference are here tabulated as follows:—



Figs. 1—5.—*Breinlia dendrolagi* n.sp. Fig. 1.—Anterior extremity. Fig. 2.—Head on larger scale. Fig. 3.—Tail of male. Fig. 4.—Tail of female. Fig. 5.—Vulva region.

Measurement.	*B. trichosuri.*	*B. dendrolagi* sp. nov.
Length of ♂ ...	10·7 cm. to 13 cm.	8 cm. to 8·3 cm.
Length of ♀ ...	18·5 cm. to 36·5 cm.	16·2 cm. to 18·2 cm.
No. of circumoral papillæ	3 pairs.	2 or 3 pairs.
Length of Oesophagus	1·84 to 2·25 (♂).	2·1 to 2·6 (♂ and ♀).
Percentage of total length	17·3% (♂).	24·3% (♂). 13·6% (♀).
Distance from tail to ♂ anogenital pore	0·60 mm. to 1·5 mm.	0·40 mm. to 0·50 mm.
Distance from tail to anus of ♀... ...	1·26 mm. to 1·73 mm.	0·28 mm. to 0·36 mm.
Length of long spicule	0·53 mm. to 0·75 mm.	0·24 mm. to 0·28 mm.
Greatest breadth of long spicule ...	0·045 mm.	0·024 mm. to 0·028 mm.
Length of short spicule	0·3 mm. to 0·33 mm.	0·080 mm. to 0·096 mm.
Greatest breadth of short spicule ...	0·045 mm.	0·024 mm. to 0·028 mm.
No. of pre-anal papillæ ... ... ♂	3 pairs.	4 pairs.
No. of post-anal papillæ ... ... ♂	3 pairs.	2 pairs.
Terminal caudal papillæ	2 pairs.	1 pair in ♀ ? 1 single in ♂.
Embryos length ...	0·18 mm. to 0·22 mm.	0·28 mm. to 0·30 mm.
Embryos, breadth...	0·003 mm. to 0·005 mm.	0·008 mm.
Perianal lips in ♂ ...	absent.	present.
Distance of nerve collar from head	0·24 mm. to 0·32 mm.	0·236 mm. to 0·256 mm.

It is thought that the points of deviation are sufficient to warrant the addition of a second species to the hitherto montypic genus *Breinlia.* The name *B. dendrolagi* n.sp. is accordingly suggested.

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Journal of Helminthology, Vol. XI, *No.* 2, *March,* 1933, *pp.* 105-108.

A New Filarial Worm from a North American Snake.

By R. K. Khanna, B.Sc., M.B., B.S., D.T.M. & H.

(*From the Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

In the course of an examination of unidentified Zoological material in the London School of Hygiene and Tropical Medicine, I recently came across a species of filarial worm from the portal vein of a North American Snake, *Coluber melanoleucus*, which I name *Macdonaldius seetai* n.g., n.sp.

The worms have a simple mouth, which is not bounded by a chitinous peribuccal ring or by epaulette-like structures ; the cuticle is transversely striated ; there are no trident like chitinous structures on each side of the anterior end of the œsophagus ; the spicules are unequal and dissimilar and the vulvar opening lies just posterior to the œsophageal region. They thus belong to the sub-family Filariinæ. Stiles, 1907.

They are of creamish white colour. The anterior and the posterior ends are attenuated. The cuticle is finely striated. The mouth is surrounded by five pairs of papillæ, arranged in three circles of two, four and four. Of the five pairs, one lies laterally and four dorso-ventrally. Figure 4 which is a free-hand drawing of the cephalic aspect of the worm indicates the position of the papillæ. The mouth leads into a small vestibule. At the end of the vestibule there is a flat ring of chitin, which lies at the extreme anterior end of the œsophagus. (Fig. 2). The œsophagus is not divisible into muscular and glandular portions.

Males.—The males are from 30 mm. to 33 mm. long with a diameter of 0·05 mm. at the anterior end and of about 0·04 mm. at the posterior end. The maximum diameter of the worm is about 0·18 mm. The œsophagus is from 0·63 mm. to 0·67 mm. long and the nerve ring is situated at a distance of 0·19 mm. from the anterior end of the œsophagus. The cervical papillæ and the excretory pore were not made out.

The posterior end of the male is coiled up into a spiral, the spicules are unequal and dissimilar and a delicate boat shaped accessory piece is present. The bigger spicule is 0·62 mm. long. It is cylindrical proximally, flattened out at the junction of the thick and the thin portions,

while its distal end is whip-like in form. The smaller spicule is 0·15 mm. long, broader proximally and shaped like a lancet at its distal end. One pair of preanal and three to four pairs of post-anal papillæ are present. The position and number of the post-anal papillæ were not constant in all the males examined. The tail is digitiform and caudal alæ are absent.

Towards the posterior end, along the ventral surface of the body, the cuticle is marked by regular transverse ridges which go half way across the body. They are especially well marked in the coiled portion of the worm and tend to decrease and disappear as the cuticle is traced proximally from behind forwards.

Females.—The females are from 65 mm. to 85 mm. long with a diameter of 0·07 mm. at their anterior end and of 0·05 mm. at their posterior end. The maximum diameter of the worm is about 0·4 mm. The length of the œsophagus is about 0·75 mm. and the nerve ring is situated at a distance of 0·21 mm. from the anterior end of the œsophagus.

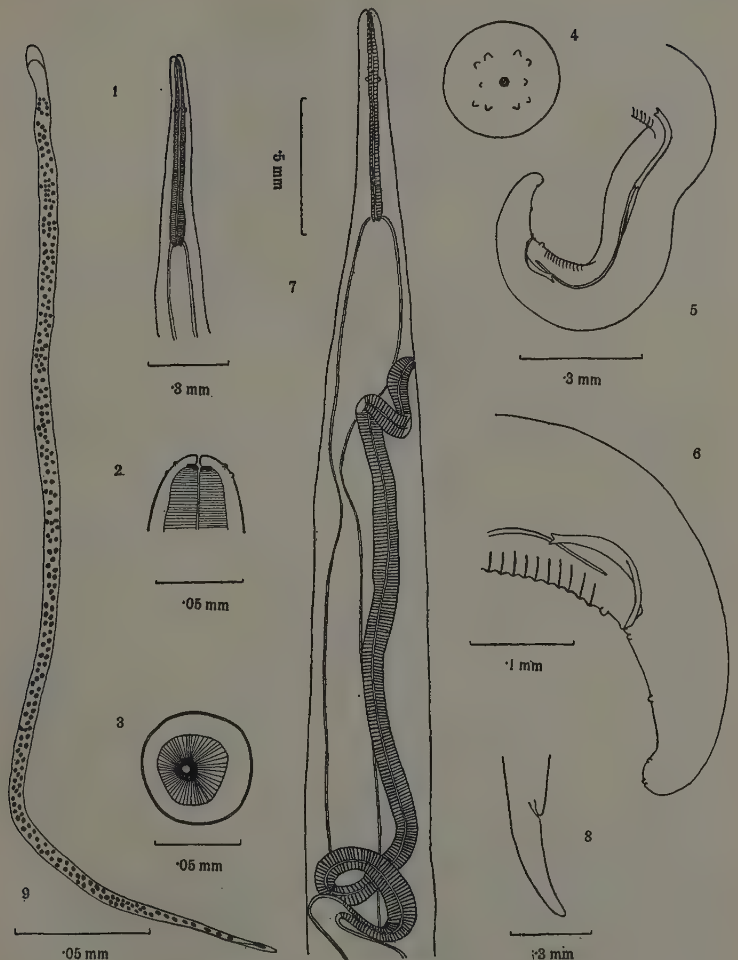
The vagina is 2·6 mm. long. It forms a loop just before opening at the vulva. The vulvar opening is situated posterior to the œsophageal—intestinal junction and is at a distance of 1·3 mm. from the anterior extremity of the worm. The anus is situated at a distance of 0·39 mm. from the tip of the tail, which in shape is bluntly rounded.

Embryos.—One mature female was dissected out and the embryos stained with Hæmatoxylin. These are sheathed and measure from 0·32 mm. to 0·39 mm. in length. The only special anatomical feature made out was the constant presence of six or seven single nuclei at the posterior end.

Discussion.

These parasites differ from the genus *Litomosa* in the following characters :—

(1) presence of a chitinous ring at the posterior end of the vestibule.
(2) absence of any enlargement at the anterior extremity.
(3) presence of head papillæ.
(4) shape of buccal cavity.
(5) presence of accessory piece.
(6) presence of striated cuticle in the male.
(7) presence of caudal papillæ in the male.



Macdonaldius seetai n.g., n.sp.

(8) shape of the spicules.

(9) absence of two small diverging processes between which are placed two minute spines at the tail end of the female.

They differ from the genus *Breinlia* in the following characters :—

(1) presence of a chitinous ring at the posterior end of the vestibule.

(2) absence of two divisions of the œsophagus.

(3) absence of any spatulate extremity in the shorter spicule in which the larger spicule glides.

(4) absence of subterminal papillæ on the posterior extremity in the female.

They differ from the genus *Hamulofilaria* in the following characters :—

(1) presence of a chitinous ring at the posterior end of the vestibule.

(2) the outline of the posterior extremity of the male.

(3) absence of a roughened callosity at the tip of the tail in the male.

(4) presence of an accessory piece.

They differ from the genus *Litomosoides* in the following characters :—

(1) presence of a chitinous ring at the posterior end of the vestibule.

(2) presence of head papillæ.

(3) shape of the vestibule, and the absence of any attachment of the anterior end of the œsophagus to the side walls of the vestibule.

(4) presence of preanal papillæ in the male.

(5) presence of accessory piece.

(6) in the sheathed character of its embryos.

The presence of a chitinous ring at the posterior end of the vestibule, is a character not found in any other genus belonging to the sub-family Filariinæ and is, in my opinion, of generic importance. The host and the position of the worm in the host also deserve notice. So far as I am aware, parasites belonging to the sub-family Filariinæ have not been described from reptiles.

I propose the generic name *Macdonaldius* for the worm.

Generic diagnosis.—Body attenuated both anteriorly and posteriorly. Mouth surrounded by five pairs of cephalic papillæ. A small vestibule at the posterior end of which there is a flat ring of chitin. Cuticle finely striated. Oesophagus not divisible into muscular and glandular portions. Parasites of reptiles.

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Journal of Helminthology, Vol. xi, *No.* 2, *March*, 1933, *pp.* 109-114.

Some Helminth Parasites from Domesticated Animals in Southern Rhodesia.

By J. J. C. Buckley, M.Sc.

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The material listed here was obtained by Dr. W. K. Blackie and Mr. W. A. McDonald during a helminthological expedition from the London School of Hygiene and Tropical Medicine to Southern Rhodesia in 1930-31. The writer is indebted to Professor R. T. Leiper for the privilege of examining and reporting on this collection of parasites. The domestic animals in question are sheep, goat, pig and cattle and although they appear to have produced no forms new to science, it is believed that the publication of this collection, inasmuch as it appears to be the only one of its kind from Southern Rhodesia, is a contribution to our knowledge of an important parasitic group.

TREMATODA.

Fam.: *SCHISTOSOMATIDÆ* Looss, 1899.

Genus: Schistosoma Weinland, 1858.

Schistosoma mattheei (Veglia and LeRoux, 1929), in sheep and cattle.

The medical importance of this parasite was discovered by the above mentioned expedition, when it was found to be harboured by man as well as by sheep and cattle. Blackie (1932) states that of 116 sheep examined, 19 or 11·3 per cent. were infected with it and of 26 cattle examined, 8 or 30·8 per cent. were infected.

Fam.: *PARAMPHISTOMIDÆ* Fischoeder, 1901.

Genus: PARAMPHISTOMUM Fischoeder, 1901.

PARAMPHISTOMUM CERVI (Schrank, 1790), in goat and cattle.

Genus inquirendum.

A few immature amphistomes, about 0·5 mm. in length, were found in a sheep.

CESTODA.

Fam.: *ANOPLOCEPHALIDÆ* Cholodkowsky, 1902.

Genus: AVITELLINA Gough, 1911.

AVITELLINA SUDANEA Woodland, 1927, in sheep.

These worms are somewhat doubtfully referred to the above species, to the description of which they approximate more closely than any of the other species described.

Genus: STILESIA Railliet, 1893.

STILESIA HEPATICA Wolffhügel, 1903, in sheep and goat.

Genus: MONIEZIA Blanchard, 1891.

MONIEZIA BENEDENI (Moniez, 1879), in cattle.

Fam.: *TAENIIDÆ* Ludwig, 1886.

Genus: TÆNIA Linnæus, 1758.

CYSTICERCUS BOVIS (=larva of *Tænia saginata* Goeze, 1782), in cattle.

CYSTICERCUS CELLULOSÆ (=larva of *Tænia solium* Linnæus, 1758), in pig.

NEMATODA.

Fam.: *RHABDIASIDÆ* Railliet, 1915.

Genus: STRONGYLOIDES Grassi, 1879.

STRONGYLOIDES PAPILLOSUS (Wedl., 1856), in sheep and pig.

Fam.: *TRICHURIDÆ* Railliet, 1915.

Genus: TRICHURIS Roederer, 1761.

TRICHURIS OVIS (Abildg., 1795), in sheep and cattle.

TRICHURIS TRICHIURA (Linnæus, 1771), in pig.

Fam.: *STRONGYLIDÆ* Baird, 1853.

Genus: OESOPHAGOSTOMUM Molin, 1861.

OESOPHAGOSTOMUM COLUMBIANUM Curtice, 1890, in sheep and goat.

OESOPHAGOSTOMUM DENTATUM (Rudolphi, 1803), in pig.

OESOPHAGOSTOMUM RADIATUM (Rudolphi, 1803), in cattle.

Genus: GAIGERIA Railliet & Henry, 1910.

GAIGERIA PACHYSCELIS Railliet & Henry, 1910, in sheep.

Genus: BUNOSTOMUM Railliet, 1902.

BUNOSTOMUM TRIGONOCEPHALUM (Rud., 1803), in sheep.

BUNOSTOMUM PHLEBOTOMUM (Railliet, 1900), in cattle.

Genus: GLOBOCEPHALUS Molin, 1861.

GLOBOCEPHALUS UROSUBULATUS (Alessandrini, 1909), in pig.

The worms which are referred to this species show certain differences in measurements from those given by Alessandrini. Thus the overall

length of these forms is greater, the male being up to 7 mm. long and the female being up to 9 mm. long, as compared with 4·4 to 5·5 mm. for the male and 5 to 7·5 mm. in the female, in the original description of *G. urosubulatus*. The spicules of the present form are longer, attaining 0·82 mm., as contrasted with 0·54 to 0·59 mm. of Alessandrini's species. Since in other respects it resembles *G. urosubulatus*, these dimensional differences alone do not appear to justify the worms being regarded as a new species.

Fam.: *TRICHOSTRONGYLIDÆ* Leiper, 1912.

Genus: TRICHOSTRONGYLUS Looss, 1905.

TRICHOSTRONGYLUS COLUBRIFORMIS (Giles, 1892), in sheep.

Genus: COOPERIA Ransom, 1907.

COOPERIA ONCOPHORA (Railliet, 1898), in cattle.

COOPERIA PECTINATA Ransom, 1907, in cattle.

COOPERIA PUNCTATA (v. Linstow, 1907), in pig and cattle.

Genus: HÆMONCHUS Cobb, 1898.

HÆMONCHUS CONTORTUS (Rudolphi, 1803), in sheep, cattle and goat.

Genus: NEMATODIRUS Ransom, 1907.

NEMATODIRUS FILICOLLIS (Rudolphi, 1802), in sheep.

Fam.: *ASCARIDÆ* Baird, 1853.

Genus: ASCARIS Linnæus, 1758.

ASCARIS LUMBRICOIDES Linnæus, 1758, in pig.

Fam. *SPIRURIDÆ* Oerley, 1885.

Genus: ARDUENNA Railliet & Henry, 1911.

ARDUENNA STRONGYLINA (Rudolphi, 1819), in pig.

Frequency of Occurrence.

With the exception of *Schistosoma mattheei*, data are not available as to the percentage infection with these parasites; in the following summary, however, the number of times that they occur in the collection is given after the name of each parasite, and may serve to some extent as a guide to their relative frequency.

Sheep.

Schistosoma mattheei, Amphistomes 1, *Avitellina sudanea* 1, *Stilesia hepatica* 2, *Strongyloides papillosus* 2, *Trichuris ovis* 1, *Oesophagostomum columbianum* 8, *Gaigeria pachyscelis* 4, *Bunostomum trigonocephalum* 2, *Hæmonchus contortus* 2, Trichostrongylus spp. 7, *Nematodirus filicollis* 1.

Goat.

Paramphistomum cervi 1, *Stilesia hepatica* 1, *Oesophagostomum columbianum* 1, *Hæmonchus contortus* 1.

Cattle.

Schistosoma mattheei, *Paramphistomum cervi* 1, *Moniezia benedeni* 1, *Cysticercus bovis* 1, *Trichuris ovis* 2, *Oesophagostomum radiatum* 5, *Bunostomum phlebotomum* 4, *Hæmonchus contortus* 3, *Cooperia oncophora* 2, *Cooperia pectinata* 1, *Cooperia punctata* 1.

Pig.

Cysticercus cellulosæ 2, *Trichuris trichiura* 2, *Strongyloides papillosus* 1, *Globocephalus urosubulatus* 1, *Oesophagostomum dentatum* 1, *Cooperia punctata* 1, *Ascaris lumbricoides* 1, *Arduenna strongylina* 3.

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Journal of Helminthology, Vol. XI, *No.* 2, *March*, 1933, *pp.* 115-118.

On *Streptovitella acadiæ* (gen. et spec. nov.). A Trematode of the Family Heterophyidæ from the Black Duck *(Anas rubripes)*.

By WILLIAM E. SWALES

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IN the spring of 1932, Mr. Otto Schierbeck, Chief Forester for the Province of Nova Scotia, forwarded several Black Ducks (*Anas rubripes*) which he had collected in a dead or dying condition on the shores of Cole Harbour, Nova Scotia, to the Animal Diseases Research Institute, Hull, Quebec. Mr. Schierbeck stated that the ducks had arrived there on their northward migratory flight six weeks earlier than usual, and after a period of hardship, had commenced to die in large numbers; and although the popular belief was that starvation was the cause of these deaths, he was not convinced that this was the case, owing to the apparently short period between the first signs of sickness and death.

The birds were examined by the author (then of the aforementioned Institute) and from the badly inflamed intestine of each bird great numbers of minute trematodes were collected. In one section, 8 microns thick, thirty-nine of these parasites were demonstrated, many of which had penetrated as far into the mucosa as the basement membrane.

The consequent study of this parasite revealed characteristics which necessitate the f ormation of a new genus, for which the name *Streptovitella* is proposed, and for the species the name *acadiæ* is proposed, in order to record the locality where the parasite was found.

Genus: *STREPTOVITELLA.*

Generic diagnosis: Heterophyinæ. Body narrow and of medium length; prepharnyx, pharynx and œsophagus well marked. Ventral sucker median, not included in a genital sac. Intestinal cæca short, not extending into the posterior fourth of the body. Ovary and seminal receptacle close together, on or near the median line. Vitellaria confined to the area behind the ovary, in a chain-like formation, following the outline of the body ventrally and meeting immediately caudad of the ovary. Ventro-genital sac contains no gonotyl, the genital opening being in the form of a simple aperture near the acetabulum.

Type and only species—*S. acadiæ* (sp. nov.).

Specific Description: This species is found deeply embedded in the intestine of birds.

Body tongue-shaped, 0·45—0·62 mm. long by 0·15—0·21 mm. wide at the testicular level. Anterior part flattened, and constricted between the intestinal bifurcation and acetabulum. Cuticle covered with small scale-like spines, these extending down to the level of the posterior extremity of the cæca. Oral sucker subterminal, 0·033—0·039 mm. in diameter; prepharynx 0·05—0·07 mm. long; pharynx sub-globular, 0·022—0·025 mm. in diameter; œsophagus 0·160—0·180 mm. long, bifurcating at a point almost half the body length from the anterior extremity. Intestinal cæca equal, fairly wide and short, 0·185—0·210 mm. in length, terminating at the anterior level of the testes.

Testes side by side, at junction of the third and posterior fourths of the body, globular to somewhat elongated in shape, subequal in size, 0·04—0·06 mm. in diameter. Seminal vesicle well developed, slightly S-shaped, contained in a powerful elongate expulsor organ, 0·065—0·070 mm. long, situated to the left of the acetabulum, somewhat overlapping that organ antero-laterally, leading to a convoluted ventro-genital sac on the right, where it unites with the end portion of the uterus near the simple genital aperture, immediately to the right of the acetabulum.

Ovary globular, 0·04—0·05 mm. in diameter on the median line, near the posterior border of the acetabulum. Seminal receptacle also globular, 0·033—0·038 mm. in diameter situated on the median line, overlapping the posterior border of the ovary (this position varies in individuals to the left of the median line caudad of the ovary). Uterus

fills all free space between the posterior level of the seminal vesicle and the lower extremity of the body.

Vitellaria in a chain-like formation, consisting of many follicles.

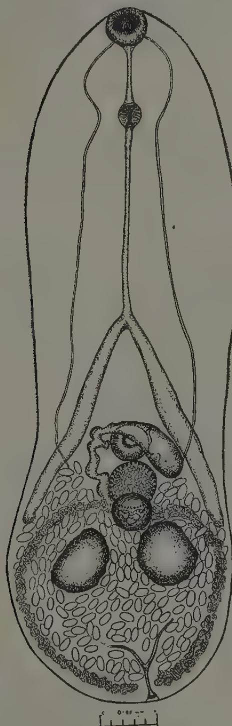

Fig. 1. *Streptovitella acadiæ* (gen. et spec. nov.). Dorsal view.

These follow the outline of the body around the lower extremity and up to the level of the testes, there curving sharply inwards and meeting immediately caudad of the ovary.

The stem of the excretory vesicle Y-shaped, the visible canals extending laterally to the oral sucker.

Eggs ovoid, 0·019—0·020 mm. long by 0·008—0·009 mm. wide, shells brownish in colour.

Host: Wild Black Duck (*Anas rubripes*).

Location: Upper part of the small intestine.

Locality: Cole Harbour, Nova Scotia, Canada.

Holotype Specimen: Helminthological Collection, Institute of Parasitology, McGill University; situated at Macdonald College, P.Q., Canada.

Paratypes deposited at:

(1) Department of Helminthology, London School of Hygiene and Tropical Medicine, England.

(1) Zoological Division, Bureau of Animal Industry, Washington, D.C., U.S.A.

Remarks: The genus *Streptovitella* resembles *Diorchitrema* Witenberg, 1928, more closely than it does any other genus of the Heterophyidæ, the outstanding distinctions being in the situation of the testes, the formation and position of the vitellaria and the relative locations of the seminal receptacle, ovary and acetabulum. Many specimens were examined before the morphology was determined as described, the variations observed being insignificant. The position of the seminal receptacle showed some variation in individuals, but the majority were consistent with the holotype.

The author wishes to express appreciation to Dr. E. A. Watson, Chief Pathologist, Animal Diseases Research Institute, Hull, Quebec, whose co-operation enabled him to make a taxonomic study of specimens collected at that Institute.

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On *Howardula phyllotretae* n. sp., a Nematode Parasite of Flea Beetles (Chrysomelidae; Coleoptera), with Some Observations on its Incidence.

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Introduction.

In the early spring of 1931 one or two hibernating adults of *Phyllotreta undulata* Kutsch., recovered from a piece of tree bark, were dissected by chance and found to be parasitized by a nematode. Realising that the host insect was not only of wide distribution throughout the country but also that, amongst farmers and other crop growers, it was known as a serious and destructive pest, this accidental record of parasitism led the writer to enter upon the present study.

Arrangements were made so that by frequent sowings a supply of food plants would be available and it finally transpired that adequate numbers of five species of the beetles were available. The parasitism of each species was investigated as well as the actual nematode itself and the results of these studies are detailed below.

Of the nematode only the stages within the body of the host beetles were encountered, viz. : the larval stages and the parasitic female stage. Although every attempt was made to find the free living stages, especially the male and female, and also the stages within the larvae and pupae of the beetles the efforts so far have not been successful. Attempts to breed the beetles along the lines indicated by Newton (1928a) failed to produce larvae and the few which were taken in the soil of the plots carrying the food plants were found, on examination, to harbour no parasites.

Through the kindness of Professor Dr. H. Blunck of the Biologische Reichsanstalt für Land- und Forstwirtschaft, in Schleswig-Holstein, a generous supply of both living and preserved specimens of *Phyllotreta nemorum* L., *P. nigripes* F. and *P. undulata* Kutsch. was sent from

Germany. This allowed a comparison between the percentage parasitism obtaining in British species with that in the German ones to be made. At the same time the writer was able to confirm the fact that the parasites present in the beetles from the two countries were identical.

Opportunity is here taken to express the writer's grateful thanks to Professor Blunck for his kindness in supplying the material so readily and for useful information concerning the parasitism of beetles found and examined by him in Germany.

An examination of the relevant literature showed that previous records of parasitism of species of *Phyllotreta* had been made but in nearly all cases the observer was content to state that nematodes occurred in the beetles without any attempt to describe the worm or treat of the bionomics, while no figures of percentage parasitism were available. Such being the case the writer has attempted, in the present study, to add to the knowledge of this nematode. The parasite appears to resemble closely *Howardula benigna* Cobb, 1921, a nematode found in the body-cavity of all stages of the Cucumber beetles, *Diabrotica vittata* (Fab.), *D. trivittata* (Mann.) and *D. duodecimpunctata* (Fab.) in the Eastern United States of America and, lacking information on the free living sexual stages, it has been considered wise, for the present at least, to place the form under discussion into the genus *Howardula*. As the writer believes the worm to be unnamed the designation of *Howardula phyllotretae* n. sp. is now proposed for it.

The Host Insects.

The parasitized insects belong to the genus *Phyllotreta*, one of several in the coleopterous family Chrysomelidae, and are popularly known under the names of " Flea Beetles," " Turnip Fleas " or " Turnip Flies." The members of this genus, like others of closely related genera, possess greatly developed powers of leaping, a distinctive character from which their popular names arise. They occur fairly commonly throughout the British Isles and are also well known in Europe where they are recognized as pests of considerable economic importance. In fact they are to be found in practically every country in the world although the British species are not always represented. Farmers, market gardeners and all who have grown cruciferous crops are well aware of the serious damage they cause which, in the aggregate, must be enormous. Not only are turnips attacked but also such valuable crops as mangolds, barley, hops, radish and even potatoes. Practically all the cultivated Cruciferae,

especially many Brassicas, as well as several common weeds suffer from attacks by *Phyllotreta*.

Of the common British species, viz.: *Phyllotreta nemorum* Linn., *P. cruciferae* Goeze, *P. atra* Payk., *P. nigripes* F., *P. undulata* Kutsch., *P. consobrina* Curt. and *P. vittula* Redt., only the first five were used in the present studies as specimens of *P. consobrina* and *P. vittula* were only encountered infrequently and not in sufficient numbers to produce reliable data concerning percentage parasitism. By far the most common form was *P. undulata* and this fact coincides with recent investigations which show that it has taken the place of *P. nemorum* which has been considered for many years as the most common and widely distributed species. The scarcity of *P. vittula* at the Institute's Farm at St. Albans is worthy of note as it is considered to be one of the chief species in Hertfordshire (Newton, 1928a).

Description and Life History.—The beetles are all minute forms, varying in size from 1·5 mm. to 3·5 mm., and are black, brassy black, metallic blue or black with irregular yellow stripes on the elytra according to the species. They are very inconspicuous on account of their small size, although the characteristic damage to the leaves of plants quickly affords a clue to their presence.

The insects hibernate as adults under tree bark, in hay stacks, hedge bottoms and under dead leaves. At the end of April and beginning of May the beetles emerge and proceed to feed on seedling turnips or other crops which may be available, or on weeds. From the end of May until August oviposition occurs, the eggs being deposited on the soil near the plants or on the leaves. As a rule the larvae, which hatch in about a fortnight, burrow into the soil and feed upon the roots although they may, at times, attack the upper parts of the plants. In the case of *P. nemorum*, however, the larvae attack the leaves of the host plants, tunnelling in the mesophyll and forming single tunnels which later become bladder-like in form. In about a month they are full-fed and descend into the soil, where pupation occurs. The adults emerge after a pupal stage lasting about two to three weeks. Pupation in the other species of *Phyllotreta* under consideration also occurs in the soil and they generally behave in all other respects in a similar manner to *P. nemorum*. After a period of feeding from the latter half of summer till about September or October they proceed to hibernate.

Damage to Host Plants.—The damage done by the *Phyllotreta* beetles is produced in two periods of attack. The most serious damage is done in the spring, when the plants are in the seedling stage, by the over-wintering generation. The beetles often appear in considerable numbers, if the weather is at all warm, and attack and totally destroy the first two leaves of the plants as they appear through the soil. Moreover, the beetles may enter the soil and eat through the fleshy hypocotyl of the developing seed and thus totally destroy the plant.

The second period of attack, in August, results mainly in leaf damage, which is less serious, and generally the crop is then able to overcome the attack by growth although the check may result in an appreciable loss in an unfavourable season.

The Parasite.

Technique.—In order to have a sufficiently large supply of material sowings of turnip and radish seeds were made on a plot of land at the Institute's Farm, at fortnightly intervals, from the beginning of April until the end of June. This provided enough host plant material in all stages of growth as food for the insects, with the result that living material was available over a wide period.

The beetles, collected by sweeping the rows of plants with an entomological net, were transferred to a suitably sized collecting tube in which they were temporarily anaesthetized with chloroform. Each specimen was carefully examined and identified specifically before proceeding to dissect it. In carrying out the dissections the insect was placed in a small quantity of Ringer's solution on a glass slide, below a binocular microscope, the elytra and wings removed by fine needles and the abdomen then gently separated from head and thorax. Infestation by nematodes was, as a rule, evident before dissection by the somewhat swollen nature of the insect abdomen and when the body was torn apart the parasites, when present, emerged and were immediately evident in the fluid. In order to ascertain the exact number of adult female worms present, the head, thorax and abdomen of each beetle was gently teased apart and the helminths removed. Frequently the larvae of the nematode were found in the body-cavity but usually in such considerable numbers that no attempt was made to count them.

For the detailed examination of the morphology of the parasites the technique adopted and detailed by Goodey (1930) was used. This consisted briefly of examination in water of specimens killed by gentle heat

from a by-pass bunsen-burner flame. The study of preserved specimens was possible after they had been fixed in hot 70 per cent. alcohol containing 3 per cent. of glycerine and allowed, by a process of evaporation, to reach finally a weak solution of glycerine. The addition of a trace of Nile blue solution after fixation to the fluid containing the specimens produced an excellent staining effect by emphasizing the details of internal structure, especially the developing gonads.

Larvae.—The larvae which issue from the vulva of the gravid female into the body-cavity of the beetle are very small and have average measurements of 0·204 mm. in length and 0·013 mm. in greatest width. Those within the uterus, especially the anterior part, of the adult female are somewhat smaller, measuring from 0·173 mm. to 0·196 mm. in length by 0·010 mm. to 0·012 mm. in greatest width.

The elongate body narrows slightly anteriorly and posteriorly, the taper being more gradual towards the rear. The head is separated from the body by a faint constriction producing a rounded knob-like appearance; no lips or papillae are discernible on the head. The terminal mouth leads into a narrow buccal tube, devoid of stylet or spear, which dilates into an elongate fusiform oesophagus, about 0·025 mm. in length, narrowing at the point where the nerve ring crosses it and thereafter slightly expanding. This oesophageal region then gives place to the intestine, which runs the length of the body, suddenly narrowing into a short rectum which leads to the indistinct anus, situated about 0·005 mm. from the tip of the bluntly rounded tail. The anus and rectum are by no means easy to identify and even from an examination of several specimens one might be led to suppose they are wanting or vestigial.

Even at this early stage within the body-cavity of the beetle the larval nematodes possess a small group of cells, the genital primordium, from which the reproductive organs develop. These cells are situated at about two-thirds of the body length from the head end and are closely applied to the intestine.

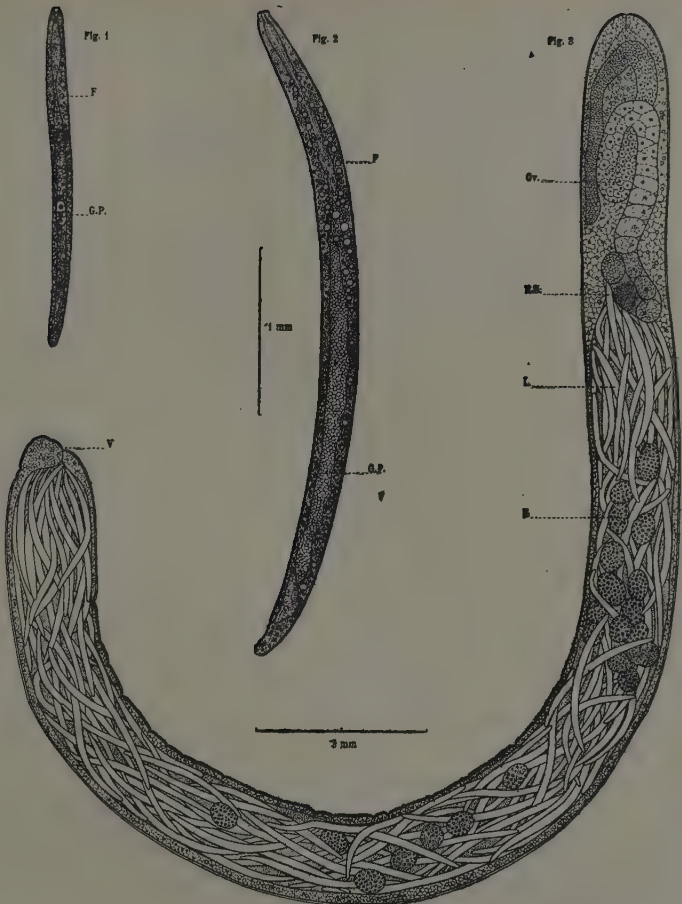
The larval food appears to consist of the fluid contents of the host's body-cavity since the worm becomes almost completely filled with globules, of varying sizes, of reserve fat material which so obscure the intestine, genital primordium and anal region that an exact perception of their shape and structure is rendered exceedingly difficult.

By a process of moulting, growth is accomplished and the larvae arrive at a stage at which no further development will take place within the body-cavity of the host. An escape to a free living condition then appears to be necessary. The larvae, prior to their escape from the beetles, have increased considerably in size and measure 0·404 mm. in length by 0·020 mm. in greatest width on the average. The general body contour remains similar to that of the early stage, but the head end becomes more defined and oesophagus larger, corresponding to the growth in size of the body. The most noticeable change in the internal morphology is the considerable enlargement of the genital primordium into a developing gonad, which now reaches a length of about 0·2 mm. and extends from about a point one-third of the body length from the head to about the level of the anus. As in the case of the early stage this larva is also packed with fat globules, which are frequently so numerous as to obscure almost completely the intestine and developing gonads.

Parasitic Female.—In contrast to the slender form of the larvae the adult female, parasitic within the body-cavity of the beetles, assumes a sausage-shape with bluntly rounded head and tail ends. In this form there is a considerable variation in size, which is dependent upon the numbers of worms present in any individual host. As a rule the greater the number of parasites present the smaller they are in size. For example a nematode unaccompanied by any others in a host insect may measure as much as 2·18 mm. in length by 0·17 mm. in greatest width. On the other hand from a host yielding 10 or more parasites their dimensions may be as small as 0·83 mm. in length by 0·063 mm. in greatest width. The average measurements computed from the examination of numerous forms are, length, 1·252 mm. and greatest width, 0·101 mm.

The body is very flaccid owing to the softening of the tissues necessitated by a parasitic life and by the great enlargement of the reproductive organs. The whole of the cuticle bears very fine transverse striations and, owing to the contraction of the body along the longitudinal axis, these striations sometimes become very pronounced, especially at the two extremities and may present an annulate appearance of the cuticle.

At the head end a minute mouth opening may be distinguished as well as a short buccal stylet lacking basal swellings. This stylet is rather obscure and difficult to identify in fixed specimens although it can be observed in living material. There is no distinct alimentary canal and only the



Howardula phyllotretae n. sp.

Fig. 1.—Larva immediately after escape from maternal uterus into body-cavity of Flea beetle. (Vertical scale.)

Fig. 2.—Larva, from oviduct of female host beetle, prior to its escape from the insect. (Vertical scale.)

Fig. 3.—Gravid parasitic female from abdominal cavity of a Flea beetle showing the greatly enlarged uterus filled with embryos and developing eggs. (Horizontal scale.)

Abbreviations : E., egg ; F., fat globules ; G.P., genital primordium ; L., embryos ; Ov., ovary and oviduct ; R.S., receptaculum seminis ; V., vulva.

cuticular anterior portion of the oesophagus is discernible. The excretory pore was not observed. At the hind end the vulva is slightly subterminal and there is no evident indication of an anus.

The bulk of the worm is occupied by the reproductive system. From the vulva the uterus stretches forward through approximately two-thirds the length of the worm. It then narrows somewhat abruptly and forms the receptaculum seminis, usually in the form of a bulbous swelling. Continuing forward this structure gives place to the oviduct which is usually reflexed. Near the head end is the narrow ovary directed forwards and turning finally in a posterior direction where it ends blindly, slightly above the level of the receptaculum seminis. In mature females the separate ova produced in the posterior portion of the ovary pass down the oviduct, through the receptaculum seminis, into the uterus. Once in the uterus the ova undergo segmentation and finally give rise to embryos. This gradual but steady production of embryos leads to a remarkable enlargement of the uterus which ultimately becomes closely packed with developing eggs and embryos. This also accounts for the position of the ovary, oviduct and receptaculum seminis which are found so far forward within the body.

Bionomics of the Parasite within the Host.—The adult female nematodes within the host were comparatively inert and only exhibited feeble movements. As a rule they were confined to the abdominal region but they were also found, not infrequently, within the thorax but never within the head. On the other hand the larvae of all stages within the beetles exhibited active movements and were encountered in the abdomen, thorax and at times in the head.

The mode of escape from the host is similar to that recorded by Cobb (1928) for *Howardula benigna* and by Bovien (1932) for *Scatonema wülkeri* in the fly, *Scatopse fuscipes* Meig. The larvae, having arrived at the stage within the body-cavity of the beetle where no further growth will occur, apparently penetrate, in the case of the female insects, into the ovaries and thence make their way to the oviduct where, on a number of occasions, they were found closely packed together in a spingle-shaped formation apparently in the act of making their exit into the soil. It seems reasonable to suppose that they are deposited along with the eggs of the beetle, as is the case in *Howardula benigna*, although no actual evidence of this procedure was obtained. In the case of the male insects which were

parasitized no specimens dissected showed any nematodes within the reproductive organs and it is not known how the escape is effected. In *Howardula benigna* Cobb found males of *Diabrotica vittata* infested, the nematodes tending to gather in the distal end of the genitalia although they were found in all parts. Experiments which he carried out to determine whether or not the worms were transferred during copulation of the insects gave negative results. Bovien is also unable to give any definite proof regarding the fate of nematodes living in the male hosts. He does suggest, however, it is probable that some of the nematodes may be able to liberate themselves after the death of the host which occurs very soon after copulation, and in moist surroundings their bodies decay in the course of a few days. On no occasion were larvae noticed in the alimentary canal of the hosts and for this reason the nematode differs in its method of escape from such forms as *Tylenchinema oscinellae*, *Allantonema mirabile* and *Bradynema strasseni*, which reach the exterior of the host *via* the anus.

Various attempts were made to induce growth in the larval forms from the oviducts of the beetles by placing them in various media such as Ringer's solution, physiological saline and others but in all cases the larvae died within a short period. With tap water similar negative results were obtained. It would seem that the difficulty encountered in attempting to culture the worms is linked up with the life history of the host. In the cases of the Frit-fly, *Oscinella frit*, and the dung fly, *Scatopse fuscipes*, both insects have a comparatively short life cycle and produce more than one generation in a year. Consequently their respective nematode parasites, in order to complete their development, also have a short life-history and growth in the various stages is moderately rapid. In the case of *Phyllotreta*, however, where, as a rule, but one generation of beetles is produced each year the growth of the various stages of the nematode is more prolonged and consequently makes it difficult to succeed with cultural methods. That they can be cultivated is, no doubt, possible but a suitable technique and medium has yet to be found for this purpose.

As mentioned in the introduction the few beetle larvae and pupae encountered were found to harbour no nematodes and the forms of the parasite which one had hoped to get in these stages of the insect were not forthcoming.

Throughout the whole course of dissection work another helminth parasite was encountered only on one occasion. This proved to be a Mermithid which was obtained from a male specimen of *Phyllotreta undulata*. The worm was an immature female and from it a specific determination was not found possible.

Ethology.

Effects of Parasitism.—Primary parasitism in insects as a rule results in the inhibition of normal development, or in reducing fecundity, or in the mortality of the host and in many instances complete sterility has been recorded. In this case the general effect seems to be a reduction both in development and in fecundity as well as a restraint upon the activity of the beetles with a consequent reduction of damage to the host plants. In the case of the allied species, *Howardula benigna*, Cobb mentions that beetles from a locality where they were not parasitized were larger, brighter and more vigorous and that, by comparison, 25 beetles from an uninfested lot were much larger and were on an average 70% heavier than a similarly chosen 25 from a 50% infested lot. He further states that " anatomical evidence shows the infested female beetles to be less fertile than the non-infested, doubt as to diminished fecundity vanishing where the female host harbours a dozen or more adult nemas. In such cases the mere relative volume of the parasites is convincing evidence of handicap."

In the case of *Phyllotreta* there was little external evidence to show the effects of parasitism. On the whole, infested insects, especially the more heavily parasitized ones, tended to be smaller and less well developed, although it was found that one was apt to be deceived since the abdomen of parasitized individuals was not infrequently swollen owing to the presence of helminths and in some cases protruded to an appreciable extent beyond the posterior limits of the elytra.

More convincing evidence as to the effects of parasitism was seen internally. In nearly all cases, irrespective of the number of parasites present, the fat-body of the insect was noticeably diminished in size and in some cases of heavy infestation it was practically non-existent.

The reproductive organs of both sexes of the insects also showed, to some extent, the effects of parasitism. In the male the reproductive organs consist of the testes, of which the investment of the follicles is

developed to the extent of enveloping them as a whole in a common coat which presents a globular appearance and is invariably of an orange colour. The two short vasa deferentia lead from this sac and unite to form a common canal, the ejaculatory duct; terminating in the aedeagus. A single pair of accessory glands originate at the point where the vasa deferentia unite to form the ejaculatory duct. These tubular glands are comparatively long and the most evident part of the reproductive system. In parasitized beetles there was no marked reduction in the size of any of the component organs of the reproductive system but the testes, in a number of cases, appeared to be very flaccid and of a colour much paler than in healthy insects. Although it was at first thought that these conditions of the testes were due to exhaustion of spermatazoa in copulation it became evident later that in some cases, at least, this was not so and throughout the entire period of collection beetles were found exhibiting this phenomenon. There was no suppression of the accessory glands such as was demonstrated by Goodey (1930) in *Oscinella frit*. The female reproductive system of the beetles consists of two ovaries each composed of ten or more ovarioles which open into the oviduct. The paired oviducts combine posteriorly to form a common oviduct which is continuous with the vagina. The bursa copulatrix is a pouch-like diverticulum of the anterior portion of the vagina and receives into its dorsal wall the very long slender duct of the spermatheca. This last organ is quite small and has a longish spermathecal gland opening into its duct. In parasitized females the spermatheca, vagina, common duct and paired oviducts were apparently unaffected but the ovaries appeared to be smaller and less well developed than in normal females owing to some inhibitory action, on the part of the parasite, to their growth. This retardation in growth or reduction in size was by no means so pronounced as in the case of the two elm bark-beetles, *Scolytus scolytus* Ol. and *S. multistriatus* Ratz., examined by the writer (1930) or in the Frit-fly as demonstrated by Goodey, and it is difficult to say with any degree of certainty that the ovaries in parasitized *Phyllotreta* females would be non-functional. It seemed reasonably evident, however, that there was a diminution in the degree of fertility and that infested insects would have a reduced capacity for egg production.

Blunk, *in litt.*, mentions that, during the course of work dealing with various species of *Phyllotreta* in Germany, *P. undulata* was very highly

parasitized by nematodes. He also mentions the fact that parasitism apparently varies in severity in different years and that he has known, in the case of *P. undulata*, of scarcely any oviposition occurring in Naumburg as well as in Schleswig-Holstein. This state of affairs resulted from the beetles being parasitized so heavily, either by nematodes or by Hymenoptera, that complete sterility or death occurred.

Incidence of Infection.—During the dissections of beetles made for the purpose of obtaining parasite material careful records were kept in order to get data concerning the incidence of infection. In order to supplement this information large numbers of insects were also preserved in 70% alcohol and dissected later. The following table summarizes the results obtained during 1931 :

British Material.		No. examined.	No. parasitized by worms.	% parasitized by worms.	No. parasitized by insects.	% parasitized by insects.
P. atra ...	♂	38	4	10·5	—	—
	♀	50	6	12·0	2	4·0
	Total	88	10	11·3	2	2·2
P. cruciferae ...	♂	38	—	—	—	—
	♀	48	2	4·1	—	—
	Total	86	2	2·3	—	—
P. nigripes ...	♂	50	7	14·0	—	—
	♀	50	3	6·0	—	—
	Total	100	10	10·0	—	—
P. nemorum ...	♂	50	—	—	—	—
	♀	50	—	—	—	—
	Total	100	—	—	—	—
P. undulata ...	♂	50	38	76·0	2	4·0
	♀	50	36	72·0	—	—
	Total	100	74	74·0	2	2·0
German Material.						
P. nemorum ...	♂	29	—	—	—	—
	♀	15	—	—	—	—
	Total	44	—	—	—	—
P. nigripes ...	♂	10	—	—	—	—
	♀	15	1	6·7	—	—
	Total	25	1	4·0	—	—
P. undulata ...	♂	36	11	30·6	3	8·3
	♀	25	6	24·0	3	12·0
	Total	61	17	27·9	6	9·8

The numbers reveal the fact that the percentage parasitism in the different species of beetles studied varies considerably. In both the British and German material the most outstanding case is that of *P. undulata* which shows considerably higher figures than any of the others. Of specimens collected at the Institute's Farm it was almost possible to say, before reliable data was forthcoming, that three out of every four beetles were infected and this is upheld by the above data. The German specimens show a much smaller percentage infestation in the neighbourhood of one in every four beetles. It is also noteworthy that the two sexes are infested practically to the same extent.

P. nigripes, of both British and German origin, and *P. atra* exhibit an infestation which, though considerably smaller than in *P. undulata*, is, nevertheless, significant. In *P. cruciferae*, on the other hand, the incidence of infection is so slight that it is doubtful whether the nematode is of any value in suppressing the pest or not. When we come to the case of *P. nemorum*, however, the entire absence of parasitism in both British and German material is rather curious.

Why there should be such great variation in the incidence of infection among the species examined is a problem which has not yet been solved but, in considering it, there are certain facts which must be borne in mind and which may have some bearing upon the problem. In the first place the lack of parasitism in *P. nemorum* may be influenced to some extent by the habits of the beetle. This is the only species which passes its larval life within leaf tissues where it mines and eats the mesophyll and is protected by upper and lower leaf epidermis. The writer satisfied himself that the larvae of *P. nemorum* did not harbour any nematodes by extracting 50 specimens from turnip leaves and 50 from radish leaves and dissecting them. In no case was any nematode found although there was a considerable incidence of parasitism by Hymenopterous insects. Larvae from turnips produced a 30 per cent. and those from radish leaves 50 per cent. infestation by Hymenopterous parasites, which would therefore seem, in the absence of nematodes, to be exercising a considerable control action.

Of the other species, the fact that *P. undulata* was by far the most common form may possibly account for its heavier infestation but this does not clear up the cases of *P. atra*, *P. cruciferae* and *P. nigripes* which were approximately equally abundant and yet show that in two cases a

10 per cent. infestation resulted, while in the third, that of *P. cruciferae*, a 2 per cent. infestation resulted.

In the case of *Howardula benigna* Cobb states that the incidence of infection in the *Diabrotica* beetles is often high and affects on the average about 20 per cent. (0%–70%) of the insects, while, on several occasions, 20 or more adult females were taken from a single beetle. The two sexes are infested about equally.

Coincident with the accumulation of data on percentage parasitism a record was kept, as each beetle was dissected, of the number of gravid female parasites in each infected host. The resulting figures are given in the following table :—

British Material.		Number of Adult Females per Individual Host.																		
		1	2	3	4	5	6	7	8	9	10	11	12	13		17		23		28
P. atra	♂	2									1	1								
	♀	3	1	1		1														
	Total	5	1	1		1					1	1								
P. cruciferae ...	♂																			
	♀	1		1																
	Total	1		1																
P. nigripes ...	♂	5			1													1		
	♀	2		1																
	Total	7		1	1													1		
P. nemorum ...	♂																			
	♀																			
	Total																			
P. undulata ...	♂	6	3	2	6	4	4	3		2	2	1	3	1		1				
	♀	7	5	6	8	2	1			2	1	3								1
	Total	13	8	8	14	6	5	3		4	3	4	3	1		1				1
German Material.																				
P. nemorum ...	♂																			
	♀																			
	Total																			
P. nigripes ...	♂																			
	♀	1																		
	Total	1																		
P. undulata ...	♂	6	1	1	1			1	1											
	♀	3	1	1	1															
	Total	9	2	2	2			1	1											

From these figures it is noticeable that one worm per host is the commonest form of parasitism and that there is a rapid decrease in numbers thereafter, except in the case of *P. undulata*, which maintains a high but gradually decreasing number of worms per host up to the extent of 11, in the British species at least. The records of 23 nematodes in a specimen of *P. nigripes* and 17 and 28 in *P. undulata* are interesting in that they show that a considerable number of parasites may be present in individual cases.

During the dissections it was observed that the parasitic female worms found in newly emerged adult beetles, not completely coloured up, were as a rule unaccompanied by parasite larvae within the host's body-cavity and that the ova within the nematode had not started to develop. As the season advanced, however, the occurrence of larvae, accompanied by adult female worms, within the body-cavity became much more frequent. No attempt was made to count the numbers of larvae present in any one host but they were encountered in many cases in hundreds and even thousands, and it is of interest to note here that Cobb (1928) removed as many as 13,000 larvae, by count, of *Howardula benigna* from the body-cavity of a single *Diabrotica vittata* and he mentions that " no doubt the number may go much higher." As the season advanced still further the numbers of larvae within the body-cavity of the beetles began to diminish until in September and October practically only adult female nematodes were found on opening up the insects. These females presumably live in the beetles during the period of hibernation and carry the infection over into the following year.

Other Nematodes Recorded in *PHYLLOTRETA*.

As already stated, an examination of the relevant literature showed that previous records of parasitism by helminths in *Phyllotreta* had been made but these furnished very scanty details. In 1920 Chittenden and Marsh mentioned the occurrence of nematodes in an American form, *Phyllotreta pusilla* Horn, the Western Cabbage Flea-beetle. Their observations are as follows :

" Nematodes infest the adult beetles. As generally observed these nematodes were young and small, about 1/40 of an inch in length. From 200 to 500 were counted in a single beetle. In several instances adult female nematodes were observed which had the body sack filled with newly developed nematode young that had not yet escaped.

"As nearly as could be determined the nematodes were not confined to the digestive tract but appeared to be in the body-cavity. In a number of cases eggs laid by the beetles were found to be infested externally with the nematodes. The eggs had an unhealthy appearance and in no instance were infested eggs observed to hatch. Just what effect the nematodes have on the beetles would be an interesting problem to work out."

The observation that the eggs, when laid by the beetles, were found to be externally infested with worms lends support to Cobb's findings in relation to *Howardula benigna*, the larvae of which are deposited from a few to upwards of fifty with each egg of *Diabrotica vittata* that is laid by infested beetles.

Pjatakova (1928) studied the species of *Phyllotreta* occurring in the Kiev Government, especially the Mleev district, during the years 1923 to 1925 and found that, besides being parasitized by various Hymenopterous parasites, additional control action was being exerted by Trombidiid larvae and nematodes. It is stated that in 1924 about 40 per cent. and in 1925 about 10 per cent. of the larvae of *P. cruciferae* were infested by an unidentified nematode. A second unidentified nematode also seems to have been observed attacking the adults of *Phyllotreta* species. An examination of the figures accompanying the text of Pjatakova's paper shows that in the case of the larval infestation (Fig. 61) the nematode is several centimetres long and seems to be a *Gordius* or *Mermis* species. The present author is inclined to the view that it is probably a species of *Mermis*, as infestation by *Gordius* worms is likely to be remote owing to the non-aquatic habits of the hosts. The other illustration (Fig. 60) shows an adult sausage-shaped form accompanied by several larvae very reminiscent of *Howardula* and apparently the specimens taken from the adult beetles.

Newton (1931) mentions that parasites, other than insects, found during life-history investigations on Flea beetles at Wye, Kent, in 1927 included a Mermithid from an adult female of *P. atra*. It was also noted in dissections by Newton that occasionally large numbers of small nematodes were present, lying free in the abdominal cavities of larvae, pupae and adults. Although unidentified it seems reasonable to suppose that these nematodes were in all probability specimens of *Howardula phyllotretae*.

Bovien (1932) also seems to be acquainted with the nematode parasite of Flea beetles for he says "That the species of flea-beetles (*Phyllotreta*)

do often harbour parasitic nematodes has been known for a long time, but strangely enough the parasites have never been described in the literature."

Diagnosis.

Howardula phyllotretae n. sp.: Parasitic stage a sausage-shaped form, more or less inert, with a buccal stylet lacking basal swellings. No distinct alimentary canal. Vulva slightly subterminal; anus none or vestigial; uterus becoming of great size, occupying greater part of body, and packed with eggs and embryos. Ovary and oviduct confined to anterior portion of body; spermatazoa localized in a swelling at end of oviduct, the receptaculum seminis. Viviparous. Average measurements: 1·25 mm. in length by 0·10 mm. in greatest width.

Parasitic in the body-cavity of Flea beetles of the genus *Phyllotreta* attacking cultivated crops and weeds in England (Hertfordshire) and Germany (Schleswig-Holstein).

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A Study of the Potato Eelworm *(Heterodera schachtii)* in the Irish Free State.

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Before dealing in detail with certain aspects of the research work on the potato eelworm which has been carried out by the present writer an account of the occurrence of the parasite in Ireland must be given.

Previous to 1922 the presence of the potato eelworm in Ireland had not been detected. On June 22nd of that year Professor Murphy, Sc.D., Mr. H. Lafferty, F.R.C.Sc.I., and Mr. T. Ward, A.R.C.Sc.I. (County Instructor in Agriculture) visited Rush, Co. Dublin (the most important early potato growing district on the east coast of Ireland) with a view to gaining some knowledge concerning a disease of potatoes which had been manifesting itself in that district for some seasons previously. The disease had come to be known locally as " flu " of the potatoes, doubtless due to the fact that it first became sufficiently noticeable to attract serious attention just about the time when the post-war epidemic of influenza was raging. As a result of the observations made on the occasion of that visit to Rush Mr. Lafferty made some notes which he has kindly placed at the disposal of the present writer. It appears from these notes that the farmers in Rush had been acquainted with the trouble in a mild form for at least ten years previous to 1922 but that it had not attracted their serious attention until a couple of years previous to that date. As these notes setting forth the observations made on the occasion of the first discovery of potato eelworm in Ireland have never been published the present writer has taken this opportunity of mentioning them.

Mr. Lafferty noted that the disease occurred in localised areas or patches of varying size and set forth a number of points which were gathered from farmers concerning the disease. These points contained references to many aspects of the disease which are now well known. He gave a good description of the appearance of the overground parts of diseased plants and the notes further stated :—" when affected plants were dug up and the underground parts examined lesions on the stem, with adhering

sclerotia of *Rhizoctonia* were common but not invariably present. Many plants were examined which to the naked eye showed no lesions or external marks whatever. In all cases, however, irrespective of *Rhizoctonia*, the roots of the plants and in many cases the rhizomes were covered with minute bodies resembling insect eggs. These egg-like bodies were approximately 0·5 mm. in diam. They varied in colour from cream to mahogany and were rather easily detached from the roots. When these bodies were examined microscopically it was at once evident that they were not insect eggs but mature female eelworms belonging to the genus *Heterodera* and each was filled with eggs. A closer inspection of the roots showed that a very considerable number of them were dead and dried up and in these dead roots live eelworms of varying sizes were seen."

On the occasion of a subsequent visit to Rush in the same year Mr. Lafferty made a careful search on the roots of other cultivated crops and a large number of different weeds but no trace of eelworm cysts could be found.

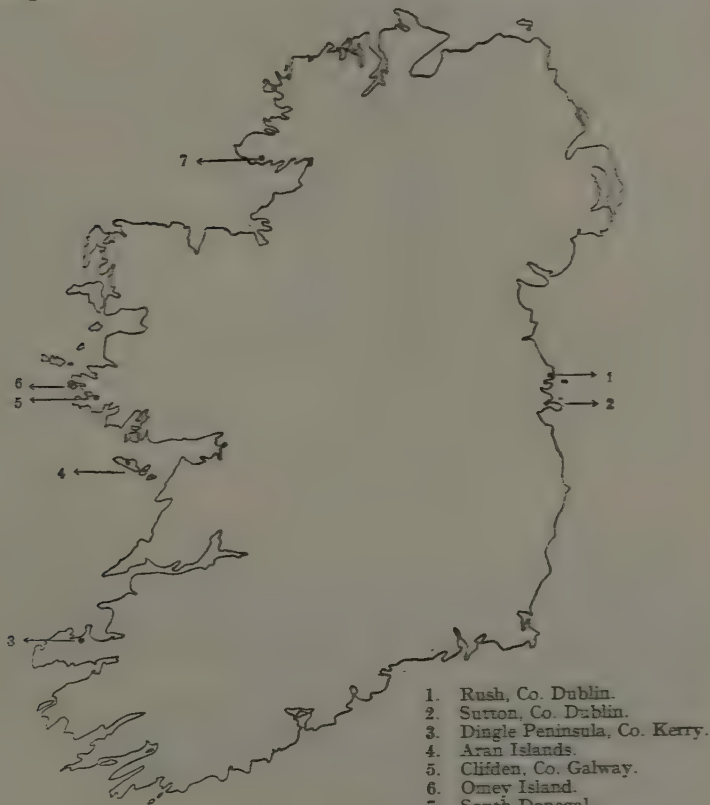
About 1925 it became known that the disease associated with eelworm infestation was present on islands off the West coast of Ireland. In 1926 Professor Murphy visited Omey Island off Connemara and also the Aran Islands with the object of making a report on the disease in these places. He found an abundance of eelworm but in practically every case the eelworm was associated with fungus diseases and an almost complete absence of rotation. As a result of his observations he concluded that the conditions of the potato crop was due to the combined effects of many causes.

In 1929 potato eelworm was discovered at Clifden, Co. Galway (on the western seaboard).

During 1930 and 1931 specimens of diseased potato plants forwarded to the Department of Agriculture for examination from the Dingle peninsula in Kerry and from the southern part of Donegal were found to have been attacked by the potato eelworm and by the fungus diseases *Rhizoctonia solani* and *Colletotrichum atramentarium*. In 1930 the present writer discovered the presence of potato eelworm in cottage gardens at Sutton, Co. Dublin. Here again there had been a complete absence of rotation for years and the plants were also attacked by fungus disease.

It will be seen, therefore, that the potato eelworm is known to occur at many places around the coast of Ireland (see map). In each of the places where it has been found the soil is of a light sandy nature. At Rush

practically the whole potato growing area is composed of blown silicious sand which forms dunes and extensive flat areas stretching in from the sea. This sand contains much carboniferous material in the form of broken and pulverised shells and is very lacking in humus. The water table is very near the surface and with suitable manuring this soil (sand) produces good crops.



The Rush district produces the bulk of the early potatoes and vegetables for the Dublin market. At Rush, eelworm is known to attack both early and late varieties of potatoes. The early varieties are planted very early in the year and are generally lifted from the last week in May onwards.

They have therefore generally made considerable growth before the hatching of eelworm eggs has become general and consequently the effects of eelworm attack does not usually manifest itself obviously on the early varieties. From about the beginning of June onwards the disease associated with eelworm attack is very obvious and widespread on second early and late varieties of potatoes at Rush.

In the gardens at Sutton where the disease occurs the soil is of much the same sandy nature as at Rush.

The following is a mechanical analysis of a typical sample of air-dried soil from Rush and Sutton respectively.

Rush.	%	Sutton.	%
Coarse sand	22·40	Coarse sand	42·50
Fine sand	65·60	Fine sand	44·40
Silt	0·25	Silt	2·00
Clay	2·75	Clay	3·25
Carbon	5·50	Carbon	1·90
Moisture	0·50	Moisture	1·20
Loss in solution	1·50	Loss in solution	1·84
	98·50		97·09
Remainder (including organic matter)	1·50	Remainder (including organic matter)	2·91
	100·00		100·00

Two fairly distinct types of soil are to be found in Aran. One type is a fairly open dark marly soil which certainly contains a fair percentage of small sized clay particles. The other type is of a very open sandy nature. This sand has a slate grey colour, having been derived from carboniferous rocks and does not contain much silicious material.

The present writer is not personally acquainted with the exact nature of the soil in other districts where potato eelworm occurs, but in each of these districts the soil is known to be of an open sandy texture.

Hatching of Eelworm Eggs.

The present writer, by experiments in 1931 and 1932, has verified the fact that hatching of potato eelworm eggs is stimulated by the presence of potato root secretion. The results of the 1931 and 1932 experiments are very similar and hence only those for 1932 are given here.

Different lots of cysts were placed in watch glasses containing tap water, potato root extract and soil extract respectively. These were then kept in an incubator at 20°C. The experiment commenced on March 1st.

Twenty cysts (two lots of ten cysts each) were placed in each medium. From the cysts in potato root extract 792 larvae had hatched up to the 16th March ; from the cysts in soil extract 80 larvae had hatched up to the 16th March ; and from the cysts in tap water 20 larvae had hatched up to the 16th March.

The number of larvae which hatched on each day was counted under a binocular microscope and at each counting the larvae were removed from the medium by means of a very finely pointed pipette.

After 16th March counts had unavoidably to be discontinued for some days. At the end of this time the larvae were so numerous in the root extract that it was not found practicable to count them. The number of larvae in the soil extract and tap water was still relatively small.

In order to obtain potato root extract a potato plant was grown in a large glass funnel filled with soil. The plant was watered regularly and the water which drained away through the leg of the funnel was collected and used as root extract.

The soil extract was obtained by putting soil in a funnel and collecting the drainage of water which was poured on to the soil.

The medium in which the cysts were kept was changed at weekly intervals.

It may be deduced from the research work on the hatching of potato eelworm eggs that under field conditions viable eggs within cysts in the soil will not hatch unless stimulated to do so by the presence of growing potatoes. It has in fact been demonstrated that eggs may remain unhatched, but viable, in bare soil for several years, and that they still possess the power of hatching if potatoes are planted in the soil. This aspect of the hatchability of eelworm eggs introduces the question of whether a rotation of crops helps in any way to control potato eelworm or to lessen the intensity of attack. From what has been said it may appear that a rotation is of little value but in actual practice it is found that such is not the case. If a field where potato sickness has been bad is kept without potatoes for three or four years and planted during that period with other crops it is generally found that such a field will then

grow a good crop of potatoes practically free from eelworm attack. The present writer has had ample evidence of this at Rush. There, a variety of market garden crop such as cabbage, lettuce, parsnips, carrots, onions, peas, etc., are generally grown in the intervening years between two potato crops and where such a practice is adopted the potatoes are kept fairly free from eelworm attack. It certainly appears that during the time while the field has been free from potatoes the eelworm eggs in the soil hatch out and the larvae, not finding a suitable crop to parasitise, die off. It is still an open question whether any or some of the crops grown during the intervening years stimulate the hatching of the eggs.

It is worthy of mention that at least two cases have been noticed by the writer at Rush where fields which were badly attacked by potato eelworm were laid down to grass for about three years. At the end of that time potatoes were again planted but contrary to expectation they were very badly attacked by eelworm and proved almost a complete failure.

One may enquire whether the eelworm could have lived on some of the plants in the pasture during the period while the ground was free of potatoes but this is hardly likely to be a satisfactory explanation. Later in this paper the question of the potato eelworm parasitising other plants is discussed.

Relationship between Intensity of Disease and pH Value of Soil.

A certain amount of research has centred around the problem of whether the incidence of potato eelworm is in any way connected with the hydrogen ion concentration of the soil. Peters (1926) investigated this problem in the hope of being able to find some correlation between the pH value of the soil and the number of cysts present (in different plots which had grown potatoes attacked by eelworm). A large number of soil samples were taken from each plot. No very definite correlationship could be established between the pH value and the number of cysts present, but the results indicated that the cysts were abundant in samples having a pH value of 6·0 (and under) and few in samples having a pH value of over 6·7. As a result of these investigations Peters concluded that a soil having a pH value of about 7·0 and over would be unfavourable to the eelworm, and suggested that the adequate liming of sour soils would probably greatly reduce the prevalence of *Heterodera schachtii*.

The present writer carried out investigations to ascertain whether a relationship could be established between the pH value of the soil and eelworm abundance. These investigations failed to reveal any such relationship and definitely disproved Peter's conclusion that a soil with a pH value of 7 (and over) was unfavourable to eelworm.

A large number of soil samples were taken from different fields at Rush and the Aran Islands. The fields from which these samples were taken had just grown potatoes which showed varying degrees of eelworm infestation ranging from negligible or only very slight infestations right up to cases in which infestation was extremely heavy with almost complete failure of the crop.

The samples of soil taken at Rush all had a pH value ranging from 7·2 to 7·55, while the samples from Aran ranged from 7·3 to 7·8. Two samples of soil taken at Sutton from a plot where eelworm infestation on potatoes was heavy gave pH values of 7·3 and 7·4. All the pH determinations were done by the Wedge colorimetric method.

It will be seen from these results that in every case, irrespective of whether eelworm was absent, scarce, or very abundant the soil always had a pH value in the narrow range of 7·2 to 7·8. It is evident therefore that no relationship between the pH value of soil and eelworm abundance can be said to exist and it is quite definite that soils having a pH above 7·0 are not unfavourable to eelworm.

In each of the districts from which samples were taken the soil contains a large quantity of broken and finely pulverised shells and hence the pH values obtained were what one would expect even by just looking at the soil.

Relationship between Intensity of Disease and Cyst Content of Soil.

It is a well known fact that one may often find areas (and perhaps whole fields) of potatoes where the plants have very many eelworm cysts on the roots and yet present a very healthy appearance. Such plants may have an abundance of normal foliage and produce a good yield of tubers. This fact has been very puzzling to research workers and it has been accountable for numerous suggestions that potato eelworm is not the cause of the well known potato sickness, or at least that it is only one of a number of contributory causes. As a result of such suggestions a certain amount of research has been carried out to ascertain whether any

relationship could be found to exist between the abundance of eelworm cysts in the soil and the intensity of potato sickness (as judged by the appearance of the foliage and roots and the yield of tubers produced). Morgan and Peters (1929) carried out some research of this nature but it appears that they were not able, by the methods they employed, to establish a relationship between the number of cysts in the soil and the intensity of potato sickness symptoms. Similarly, research by Smith and Prentice (1929) gave on the whole negative results although there was an indication from their work that " where ' eelworm disease ' has been noted recently there is a positive association of the intensity of disease and cyst content of the soil."

The negative results obtained by Morgan and Peters, and by Smith and Prentice do not, by any means, indicate conclusively that potato eelworm is a parasite of minor importance. These workers do not seem to have taken into serious account such variable factors as natural fertility of the soil and type of manuring ; quality of seed used ; date of planting and whether tubers were sprouted or not ; amount of growth and root development before hatching of eelworm eggs in the soil could have commenced to any extent ; condition of plants when they were first attacked by eelworm, etc. Moreover, they used for their cyst count determinations soil obtained after the crop (about which they made notes as regards potato sickness symptoms) had been lifted. Doubtless it would have been much better if the cyst content of the soil had been determined before the crop was planted. Miles (1930) carried out some research in which he made counts of the cyst content of the soil both before planting and after lifting the crop but his results are also of a more or less negative nature.

The present writer has endeavoured to ascertain whether the number of eelworm cysts in the soil before the crop is planted can be correlated with the intensity of potato sickness symptoms. The method by which this was done is described later. The cyst count determinations were made according to the flotation method devised by Morgan (1925).

The pot experiments described in this paper were carried out at the Albert Agricultural College, Glasnevin, in 1932. For these experiments soil from potato sick land at Rush and Sutton was used and also soil of a sandy nature obtained from a grass field (at Howth, Co. Dublin) which had never grown potatoes during living memory. The following table

explains how the pots were arranged in different series and the table is followed by a detailed description of how the experiments were carried out.

Series.	Serial No. of pots.	Treatment of soil used.
I (Using potato sick soil from Rush)	1 and 2	Untreated (as obtained from field).
	3 ,, 4	Steam sterilized.
	5 ,, 6	Steam sterilized (same as 3 and 4) and afterwards infected with cysts at the rate of 3 *cysts* for *each c.c.* of soil in pot.
*II (Using potato sick soil from Rush)	1 and 2	Untreated (same pots as in Series I).
	7 ,, 8	Soil sieved through a sieve containing 100 wires to the inch and fine portion used for experiment.
	9 ,, 10	Sieved (same as 7 and 8) and afterwards steam sterilized.
	11 ,, 12	Sieved (same as 7 and 8) and afterwards infected with cysts at the rate of 3 *cysts* for *each c.c.* of soil in pot.
		*The sieved soil was in each case restored to something like its original consistency, before being potted, by the addition of sterilized silver sand.
III (Using potato sick soil from Sutton)	13 and 14	Untreated (as obtained from field).
	15 ,, 16	Steam sterilized.
	17 ,, 18	Steam sterilized and afterwards infected with cysts at the rate of 1 *cyst* for each 4 *c.c. of soil* in pot.
	19 ,, 20	Steam sterilized and afterwards infected with cysts at the rate of 1 *cyst* for *each c.c. of soil* in pot.
	21 ,, 22	Steam sterilized and afterwards infected with cysts at the rate of 4 *cysts* for *each c.c. of soil* in pot.
IV (Using soil that had never previously grown potatoes)	23 and 24	Untreated (as obtained from field).
	25 ,, 26	Untreated but infected with cysts at the rate of 1 *cyst* for *each* 4 *c.c. of soil* in pot.
	27 ,, 28	Untreated but infected with cysts at the rate of 1 *cyst* for *each c.c. of soil* in pot.
	29 ,, 30	Untreated but infected with cysts at the rate of 4 *cysts* for *each c.c. of soil* in pot.

Details of Procedure in Conducting Pot Experiments.

Preparation of Soil.—The soil used in the experiments was brought in from the field in the Autumn of 1931 and kept in sacks in a store until required in the spring of 1932. By that time it had become well air dried.

Sieving of Soil.—Soil from Rush was chosen for sieving because, on account of its texture, the operation could be performed with very much greater ease than if soil from Sutton was used. Sieving was done after the soil had reached an air dried condition. It was assumed that the fine soil which passed through the sieve would be free from eelworm cysts but that fungus spores would not be removed. It was further assumed that any natural soil factors would not be altered except in so far as the removal of the coarser particles might have an influence. After the sieving operation had been performed a quantity of sterilized silver sand, equal to the amount of coarse material removed by sieving, was added in order to restore the sieved soil to something like its original natural consistency.

A microscopical examination of the sieved soil revealed the fact that a small number of very tiny cysts had passed through the sieve. Many of these cysts were carefully examined but as far as could be determined each seemed to be devoid of eggs. It was recognised, however, that some of them might possibly contain a few eggs and moreover that free eggs which may have been liberated from cysts (*e.g.*, by breakage of cysts) could have passed through the sieve.

Sterilization.—The sterilization of soil (and leaf mould) was performed in an autoclave about the first week of March. The sterilized soil was then potted up immediately and the setts planted about a month afterwards.

Collection of Eelworm Cysts.—It was anticipated that the collection of a sufficient quantity of cysts, for the experiments described in this paper, would be a rather tedious operation and accordingly provision was made during the previous season for facilitating the collection of these cysts. In 1931 a large number of pots were prepared with potato sick soil from Rush and planted with potato setts. The plants which grew in these pots were heavily attacked by eelworm and an abundance of cysts developed on the roots. About July the pots were turned out and in most cases the roots had formed a mat of fibres, densely covered with cysts, around the mould of soil. This mat of roots was removed with a scissors and forceps and thus a large quantity of cysts were collected on the root material, free

from soil. When this root material had dried it was rubbed lightly in order to detach the cysts, and then sieved. The cysts passed through the sieve and were thus obtained contaminated only with the smaller particles of root material and particles of foreign matter. This cyst material was then placed, in small quantities at a time, on a large sheet of strong drawing paper. The paper was tilted and tapped quickly and lightly. This tapping caused the cysts (which are very smooth surfaced) to roll away quickly from the remainder of the material and thus they were collected practically uncontaminated with foreign material. Afterwards the pieces of foreign material were picked out under a binocular microscope.

The cysts thus collected were kept in a dry condition until the spring of 1932. About February, five lots of cysts, each of ·05 gms. were weighed and the number of cysts in each lot was counted. It was found that each lot of ·05 gms. contained, on an average, about 2,000 cysts (1,970 was the figure actually obtained). This result was made use of in determining the weight of cysts which should be used in order to infect the different pots at the required rates.

" Seed " Tubers.—" Seed " tubers of the variety " Up-to-Date," raised from plants free from leaf roll and mosaic diseases (" certified seed ") were procured for the experiment. These tubers were put in ·001% solution of mercuric chloride for 15 minutes and afterwards washed with tap water. They were then stored in sprouting boxes until required for planting.

Preparation of Pots.—Nine-inch porous flower pots were used. The different materials for each pot were measured out accurately in order to eliminate variability between the pots as much as possible. Each pot was made up so as to contain 4,000 c.c. of soil, ½ lb. of well rotted sterilized leaf mould and 3 gms. of mixed inorganic fertiliser. The latter ingredient was made by mixing 3 parts (by weight) of xxx superphosphate, 1½ parts of sulphate of ammonia and 1½ parts of muriate of potash. The dressing of 3 gms. per pot was calculated to represent a dressing of about 8 cwt. per statute acre.

The ingredients for each pot were well mixed together on a sheet of paper and in all cases where the soil had to be infected with eelworm cysts these were added and mixed with the other ingredients. Particular care was taken when mixing to prevent accidental contamination of sterilized or sieved soil with eelworm cysts (*i.e.*, in cases where no cysts were being added).

The pots were prepared as described above early in March and during the first week of April a fairly well sprouted tuber was planted in each pot. All the tubers used were about the same size and each generally had three sprouts. After the tubers had been planted the pots were sunk in the soil, out of doors, to within about two inches from the rim. This lessened the loss of water from the pots and reduced the necessity of frequent watering. During the growing period the pots were watered as often as was considered necessary and weeds were removed.

Results of Experiments.

The plants began to appear above the soil within a short time after the tubers had been planted. During the growing period they were closely watched and many observations were made as regards growth and appearance. These observations are summarised in notes which are given later.

The photographs presented herein were taken during the first week of July. It was necessary that they should be taken then on account of the fact that plants showing severe symptoms of potato sickness would not remain alive much longer. About the middle of July the pots were turned out in order that the roots might be examined and the weight of tubers ascertained. It should be borne in mind that if the healthy plants had been allowed to continue growth the yield of tubers from them would have been much greater, whereas the yields given for the obviously diseased plants represent the maximum limit of production.

In studying the photographs and yields, reference should be made to the table already given. In each case the serial numbers of the pots and the combined yield of the two pots are given at the side.

Notes on Plants in Different Series.

Series I.

Pots 1 *and* 2.—These plants remained stunted and exhibited typical severe symptoms of potato sickness. The roots were poorly developed and unhealthy looking and eelworm cysts were abundant. Both *Rhizoctonia solani* and *Colletotrichum atramentarium* were present also but as well as could be judged only as a slight infestation.

SERIES I. SERIES II.

(a)

(b)

(c)

(d)

(e)

(f)

(g)

Series I Using potato-sick soil from Rush.

Series II Using potato-sick soil from Rush.

Series I (*a*) 1 and 2 soil in natural condition (5 cysts per c.c.) yielded 1¼ ozs.
(*b*) 3 and 4 soil sterilised yielded 14¾ ozs.
(*c*) 5 and 6 soil sterilised, with 3 cysts per c.c. subsequently added, yielded 9½ ozs.

Series II (*d*) 1 and 2 soil in natural condition (Series I (*a*) repeated).
(*e*) 7 and 8 soil seived yielded 11½ ozs.
(*f*) 9 and 10 soil sieved and subsequently sterilised yielded 12½ ozs.
(*g*) 11 and 12 soil sieved, with 3 cysts per c.c. subsequently added, yielded 4¼ ozs.

(To face page 148.)

SERIES III.

SERIES IV.

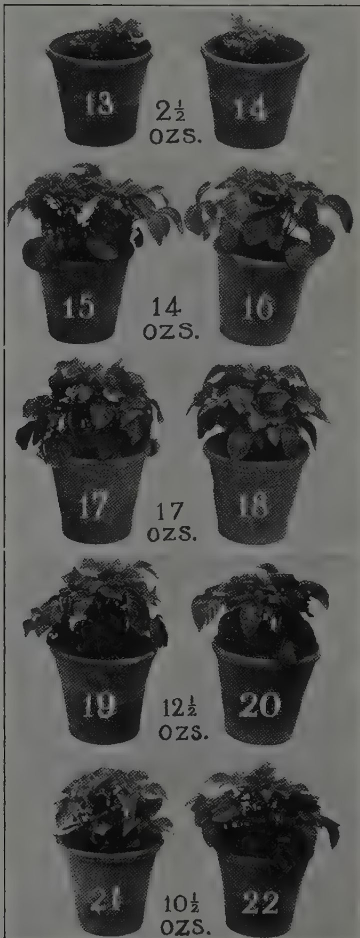
(h)

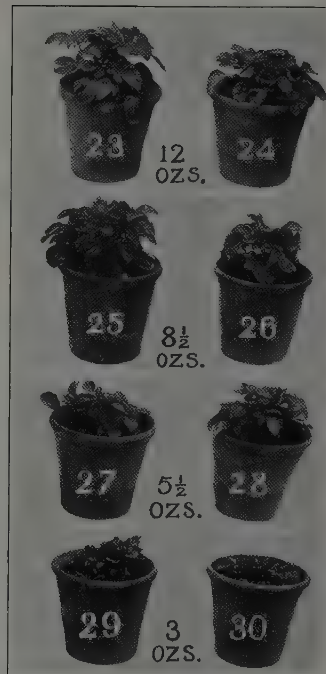
(i)

(j)

(k)

(l)





(m)

(n)

(o)

(p)

Series III Using potato-sick soil from Sutton.

Series IV Using soil that had never previously grown potatoes from Howth.

Series III (h) 13 and 14 soil in natural condition (2 cysts per c.c.) yielded 2½ ozs.
(i) 15 and 16 soil sterilised yielded 14 ozs.
(j) 17 and 18 soil sterilised, with 1 cyst per 4 c.c. subsequently added, yielded 17 ozs.
(k) 19 and 20 soil sterilised, with 1 cyst per c.c. subsequently added, yielded 12½ ozs.
(l) 21 and 22 soil sterilised, with 4 cysts per c.c. subsequently added, yielded 10½ ozs.

Series IV. (m) 23 and 24 soil in natural condition yielded 12 ozs.
(n) 25 and 26 soil, with 1 cyst per 4 c.c. added, yielded 8½ ozs.
(o) 27 and 28 soil, with 1 cyst per c.c. added, yielded 5½ ozs.
(p) 29 and 30 soil, with 4 cysts per c.c. added, yielded 3 ozs.

Pots 3 *and* 4.—These plants grew to a good size and remained perfectly healthy looking. The roots were well developed and healthy. There were no fungus organisms present and no eelworm cysts.

Pots 5 *and* 6.—As can be seen from the photographs these plants grew to almost as good a size as Nos. 3 and 4 but appeared to be less sappy. Apart from this they remained quite healthy looking throughout the period of growth. The roots appeared to be almost as healthy and well developed as the roots of Nos. 3 and 4 but there were a large number of eelworm cysts present and the yield was appreciably reduced.

It must be remembered that the soil in which these plants were grown contained only 3 cysts per c.c. whereas the untreated soil in pots 1 and 2 contained about 5 cysts per c.c.

Series II.

Pots 1 *and* 2.—See Series I.

Pots 7 *and* 8.—One of these plants (No. 8) grew to a good size and remained perfectly healthy looking. Its roots were well developed and sound. A certain amount of *Colletotrichum atramentarium* was present but the effect on the roots seemed to be very slight. The yield of this plant was 7½ ozs.

Plant No. 7 remained low sized (see photograph) but apart from this it maintained a very sappy and healthy appearance throughout its period of growth. Its roots were well developed and healthy and no fungus organisms could be found. The yield of this plant was 4 ozs.

On the roots of both plants there was a very slight infestation of eelworm cysts. The manner in which this infestation may have come about has been already discussed.

The combined yield of both plants (11½ ozs.) was only very slightly under the yield from pots 9 and 10 (sterilized) and if plant No. 7 had been as large as No. 8 the total yield would certainly have been higher.

Pots 9 *and* 10.—Good sized healthy plants with well developed roots free from eelworm cysts and fungus organisms.

Pots 11 *and* 12.—Both these plants remained low sized and showed typical symptoms of potato sickness. They were not as bad as the control pots (Nos. 1 and 2) but it must be remembered that these pots did not contain as many cysts per c.c. as did the controls. The roots were poorly

developed, distinctly unhealthy and had a fairly heavy infestation of eelworm cysts. There was a fair amount of *Rhizoctonia solani* on the roots of plant No. 11 but the amount of fungus on No. 12 was negligible.

Series III.

Pots 13 *and* 14.—These plants showed typical severe symptoms of potato sickness. The roots were poorly developed, very unhealthy and had a fairly heavy infestation of eelworm cysts. On No. 13 there was a large amount of *Rhizoctonia solani*. On No. 14 there was a fair amount of *Rhizoctonia* and also some *Colletotrichum atramentarium*.

Pots 15 *and* 16.—Good sized healthy plants with well developed sound roots free from eelworm cysts and fungus organisms.

Pots 17 *to* 22.—All these plants grew to a good size and maintained a perfectly healthy appearance throughout the period of growth. In all cases the roots were healthy, well developed and free from fungus organisms.

Nos. 17 and 18 had a slight infestation of eelworm cysts on the roots but the yield from these pots was somewhat higher than from Nos. 15 and 16 (where no cysts were added).

Nos. 19 and 20 had a fair infestation of eelworm cysts and the yield was reduced.

Nos. 21 and 22 had a fairly heavy infestation of eelworm cysts and the yield was further reduced.

Series IV.

Pots 23 *and* 24.—These plants grew to a fairly good size and remained healthy looking throughout the period of growth. The roots were well developed and healthy but there was a little *Rhizoctonia solani* present. No *Colletotrichum atramentarium* was seen and there were no eelworm cysts.

Pots 25 *and* 26.—These plants grew to a fair size but they were less sappy looking than 23 and 24 and showed certain signs of potato sickness. They started to turn yellow and wither off prematurely. The root development was fairly good but a certain amount of root decay was noticeable. There was a slight infestation of eelworm cysts on the roots

and also a little *Rhizoctonia solani* but no *Colletotrichum atramentarium*. The yield was appreciably lower than from 23 and 24.

Pots 27 *and* 28.—These plants remained low sized and exhibited obvious symptoms of potato sickness. The roots were poorly developed and root decay was quite noticeable. There was a fairly heavy infestation of eelworm cysts and a little *Rhizoctonia solani* but no *Colletotrichum atramentarium*. The yield was further reduced.

Pots 29 *and* 30.—These plants remained very dwarfed and exhibited severe symptoms of potato sickness. The roots were very poorly developed and unhealthy and had a heavy infestation of eelworm cysts. There was a little *Rhizoctonia solani* on both plants and a fairly heavy infestation of *Colletotrichum atramentarium* on one of them. The yield was still further reduced.

Discussion of Results.

In discussing the results of the above experiments the series will be considered in the order IV, II, I and III.

In series IV in which soil that had never previously grown potatoes was used, it can be seen that the soil in its natural condition was capable of producing good potato plants free from obvious disease symptoms. It is true that there was a little *Rhizoctonia solani* on the roots of these plants but the presence of this fungus did not appear to have much effect on the health of the plants. The inoculation of this soil with eelworm cysts did give rise to symptoms of potato sickness. A glance at the photographs in this series and a study of the notes *re* the plants shows that the symptoms of potato sickness (both overground and underground) were exactly proportional to the degree of infestation with eelworm cysts.

It appears obvious therefore that potato sickness is caused by the attack of the potato eelworm and that the intensity of the disease is influenced very much by the number of eelworms present in the soil.

In series II, soil containing a natural infestation of potato eelworm was used. It was sought to remove the eelworm factor, and only the eelworm factor entirely, from this soil without altering the soil in any other way. It was assumed that this could be done without sterilization by a sieving process which has been already described. The fine soil which passed through the sieve would still contain spores of fungus organisms which

may have been present and would also possess any peculiar inherent characteristics or soil factors which are often mentioned in a vague way in publications dealing with the potato eelworm. It has already been mentioned that the fine sieved soil was subsequently restored to something like its original degree of coarseness by the addition of sterilized silver sand, and this should have rectified any difference which may have resulted by removing the coarser particles from the soil. Pots 7 and 8 in this series demonstrate satisfactorily that the cause of potato sickness can be removed from the soil by sieving it in such a way as to remove eelworm cysts. It is true that for some unexplained reason, plant No. 7 remained low sized but still this was a perfectly healthy plant which did not show any symptoms of potato sickness. The plants which grew in sieved soil which was subsequently sterilized (Nos. 9 and 10) were in no way better than plant No. 8. Plants 11 and 12 demonstrate clearly that soil which has been freed from potato sickness by sieving can be again made potato sick by adding the cysts of potato eelworm to it.

The results obtained in this series substantiate the results obtained in series IV in so far as they make it appear obvious that potato sickness is caused by the attack of potato eelworm. The experiments in this series were not sufficiently exhaustive to indicate whether there is a relationship between the intensity of the disease and the number of eelworms present in the soil.

The results obtained in Series I and III do not verify the conclusions arrived at from Series II and IV. In Series I and III it is clearly shown that the cause of potato sickness can be removed by steam sterilization of the soil. The addition of eelworm cysts, however, to such sterilized soil did not cause the reappearance of typical symptoms of potato sickness, although the resulting infestation of eelworm cysts on the roots of the plants was approximately proportional to the number of cysts added to the soil. Plants 5 and 6 in Series I did, however, appear less vigorous and less sappy than the plants grown in sterilized soil free from cysts.

It appears from Series I and III that steam sterilization in addition to killing the eelworm eggs (and fungus organisms) has many other effects on the soil which are not clearly understood. The sterilization seems to alter the soil to such an extent as to cause it to grow, or enable it to grow, good healthy potato plants despite the presence in the soil (as a result of inoculation) of large numbers of potato eelworms. The presence of cysts

on the roots of the plants indicates that the plants are actually attacked by the eelworm but nevertheless symptoms of potato sickness did not appear.

The experiments carried out by the present writer were not sufficiently exhaustive to indicate why potato eelworm was not able to produce typical symptoms of potato sickness in soil which had been sterilized. Somewhat similar experiments recently carried out in England by Buckhurst and Fryer (1931) gave in the first year results of the same nature as those obtained in the experiments of Series I and III described in this paper. Buckhurst and Fryer allowed the pots to remain as they were and replanted them with potatoes in the second year. It was then discovered that the plants in pots containing potato eelworm showed typical symptoms of potato sickness, which result seemed to indicate that by the second year the effects of the previous year's sterilization had worn off.

The present writer hopes to carry out further experiments with a view to elucidating some of the problems connected with sterilization of the soil and ascertaining how long the effects of sterilization persist.

Soil Treatment.

A certain amount of research on the treatment of soil with different substances, for the control of potato eelworm, has been carried out in Great Britain and elsewhere. Experiments carried out by Edwards (1929) in Lincolnshire in 1927 and 1928 indicated that a good control of eelworm had been obtained by dressing the soil with Creosote Salts (crude naphthalene which contains small quantities of coal tar derivatives).

The present writer carried out extensive field trials with this material both at Rush and the Aran Islands in 1930 but obtained no degree of control whatsoever. Also, the results obtained from extensive pot experiments confirmed the observations made in the field as regards the inefficiency of Creosote Salts for controlling potato eelworm.

Pot experiments to ascertain whether other substances may be of use as soil dressings have also been carried out by the present writer, the following substances being tested :—naphthalene, para-di-chlor-benzene, cresylic acid emulsion, carbon bisulphide, copper sulphate, potassium permanganate and calcium cyanimide. Negative results were obtained with all these except calcium cyanimide. The plants which grew in the

pots treated with calcium cyanimide made much better growth than the control plants in the untreated soil. They did not exhibit such severe overground symptoms of potato sickness; the root development was better; the yield was greater and there was an appreciable reduction in the number of cysts on the roots. These plants of course received much more nitrogenous fertiliser than the control plants because of the additional calcium cyanimide and this may have accounted for a certain amount of the difference. Further, more extensive and more exhaustive tests with calcium cyanimide are desirable before very definite conclusions can be drawn.

Different Strains of *Heterodera Schachtii.*

It is now known that many cultivated crops and weeds may be attacked by different biological strains of *Heterodera schachtii*. Certain strains have been carefully studied by research workers with the object of ascertaining whether they could attack, or in the course of time become adapted to, species of plants on which they do not normally occur. This research has shown the various strains to be very specialised but there are many cases on record where certain strains have gone over on to new host plants after having been in close association with these plants for some time (generally a number of years).

The present writer does not know of a single instance where a cultivated crop, other than the potato, has been attacked by *Heterodera schachtii* in Ireland. Although table beet is grown very extensively at Rush in close association with potatoes the parasite has never been found on that crop. The only weed plant on which the present writer has ever found *Heterodera schachtii* is the curled dock. It was discovered on docks at Rush in 1930 and observations since then have revealed that the parasite is common on these plants at Rush. The parasite has also been found on docks both on the Aran Islands and at Sutton in close proximity to where potato eelworm occurs, but it has not been found on these plants elsewhere. This would seem to suggest that there is some association between the dock strain and the potato strain of the eelworm, but up to the present all attempts made by the writer to infect potatoes with the dock strain or to infect docks with the potato strain have been failures.

Acknowledgments.

At different stages during the course of the research outlined in this paper the writer has been ably assisted by E. McMahon, B.Agr.Sc., J. Brady, B.Agr.Sc., and T. Turpin, B.Agr.Sc. This opportunity is taken to acknowledge the help received from them and also to thank Professor P. A. Murphy, Sc.D., for the information which he has given in connection with the history of the potato eelworm in Ireland, and for having examined experimental plants for fungus organisms.

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The Helminth Parasites of Dogs in Marseilles.

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Dogs are notoriously susceptible to helminthic infections, especially of the alimentary tract. Their rôle as carriers of such important pathogenic organisms as *Echinococcus* and *Taenia multiceps* makes the study of their parasites of more than academic importance, so much so that by an order of the United States Bureau of Animal Industry, all sheep dogs imported into the U.S.A. have to be held in quarantine pending a faecal examination for helminthiasis (Wigdor, 1919). Statistical surveys of the helminth parasites carried by dogs have already been made in a number of different countries. In this country the subject has been studied by Lewis at Aberystwyth, in Wales; and by Nuttall and Strickland in Cambridge. Brown and Stammers have made a parasitological survey of the dogs in London based on post-mortems combined with examination of faeces from London pavements.

On the Continent there exists the authority of Krabbe for Denmark and Iceland, Skrjabin for Russia, Deffke for Berlin, etc. In America there have been the surveys of Sommer for Washington, D.C., and of Hall for Detroit, Mich. As regards the tropics, surveys have been made by Wharton in the Philippines and by Gordon and Young in Mañaos, Brazil. Since, so far as I have been able to discover, no such survey has been published dealing with dogs in the South of France, it was thought worth while to record the following observations made in the course of 37 post-mortems carried out on stray mongrels from the town and district of Marseilles.

I take this opportunity of expressing my gratitude to Professor Joyeux of the Marseilles Medical College for suggesting and supervising the work and for providing me with laboratory facilities; and also to Professor Mercier, of the Department of Physiology, for providing me with the dogs after his class had finished with them.

None of the 37 dogs examined was entirely free from parasites. In each case the following organs were examined :—heart, liver, lungs and bronchi, and alimentary canal from stomach to rectum. In most cases

blood and mucus smears from the bronchi and faecal smears from the rectum were made and examined for helminth ova. The oesophagus and stomach were examined for *Spirocerca* nodules and the heart for *Dirofilaria* without result.

A single embryonated ovum (Nematode) was found in a bronchial smear. Unfortunately the egg was lost and no further eggs or worms were found in the lungs of this dog, but it is thought that this might be a case of *Haemostrongylus vasorum*, a Metastrongyle occurring in the respiratory and circulatory system of carnivores. The liver was in all the cases examined healthy and free from cysts ; as was also the spleen except for a single, large splenic haematoma which was not parasitic.

Examination of the faecal contents of the rectum convinced the writer that a negative finding is not a reliable indication that the dog is free from parasites unless the examination is kept up over a considerable period. Immature infections, or infections consisting only of males or of unfertilised females (in the case of Nematodes), frequently cannot be demonstrated by faecal examination.

Certain intestinal parasites common in dogs elsewhere were not found in Marseilles. Thus Sommer (1896) records *Trichuris depressiusculus* (= *T. vulpis*) as the commonest parasite of dogs in Washington (70%). This was never present in the dogs examined by me. *Ancylostoma caninum* which is, with *A. braziliense*, the dominant hookworm of dogs in the tropics (Wharton, 1917, Gordon and Young, 1922), was also absent, though Joyeux has found it in a dog from Corsica. No Trematode parasites of any kind were found. *Taenia multiceps* (= *Multiceps multiceps*) has been recorded from dogs at Toulouse (Bailliet, 1863), but was not present in any that I examined in Marseilles.

The parasites found comprised the following :—*Toxascaris leonina*, *Toxocara canis*, *Dochmoides stenocephala* (adult and immature worms), *Echinococcus granulosus*, *Taenia pisiformis*, *Taenia hydatigena*, *Mesocestoides lineatus*, and *Dipylidium caninum*. It was noticed that in no case were either *Taenia pisiformis* and *T. hydatigena* ; or *Toxascaris leonina* and *Toxocara canis*, present in the same dog. This suggests that infections such as these may render the dog refractory to subsequent infections with closely related worms. On the other hand one dog showed both adult and very young specimens of *Taenia pisiformis*, clearly demonstrating that though the dog already carried a moderate infection, this did not

render it immune to a subsequent infection with the same parasite. The greatest number of species found in a single dog was five ; this dog being infected with *Toxascaris leonina*, *Taenia pisiformis* (adults and immature worms), *Dochmoides stenocephala* (adults and immature worms), *Echinococcus granulosus* and *Dipylidium caninum*.

NEMATODES.

Toxocara canis (Werner, 1782) (= *Belascaris marginata* (Rud.)).

This ascarid was found in five dogs or 13·5% of those examined. Not more than a dozen worms were ever found in one dog.

Toxascaris leonina (von Linstow 1902) occurred in 7 dogs or 18·9%. The greatest number in any one dog was about 12 or 14.

Dochmoides stenocephala (Railliet, 1884)
(= *Uncinaria stenocephala* (R. 1884)).

This hookworm was present in five of the dogs examined, *i.e.*, in 13·5%. It occurs mainly in the upper part of the intestine and is very firmly attached to the mucosa. The infections varied from a single worm to about 40 specimens. In all cases other species of worms were present.

CESTODES.

Bailliet (1863, p. 453) describes six species of adult Cestodes occurring in the dog at Toulouse, viz. : *Taenia pisiformis*, *T. multiceps*, *T. hydatigena*, *Dipylidium caninum*, *Echinococcus granulosus* and *T. pseudo-cucumerina*. From the description which Bailliet gives, this last is almost certainly *Mesocestoides lineatus*. All of these were present in the Marseilles dogs except *Taenia multiceps*.

Taenia pisiformis (Bloch 1780) Gmelin, 1790. (= *T. serrata*).

Found in 15 dogs or 40·5%. Two of these infections consisted entirely of very young worms and one dog as already mentioned showed an old and a young infection.

Taenia hydatigena (Pallas 1766). (= *T. marginata*).

This tapeworm was found only in four dogs (10·8%). Apparently *T. pisiformis* is about four times as common as *T. hydatigena* in Marseilles.

Echinococcus granulosus (Batsch 1786) Rudolphi 1805.

As was the case with Lewis (1927), I was struck by the relative rarity of the adults in a region where the larva is reputed to be abundant. It is said that nearly 100 per cent. of the sheep brought to Marseilles for

slaughter from the Camargue district at the mouth of the Rhône, are infected with hydatid cyst. The writer saw heavy pulmonary and hepatic infections in sheep offal at the Marseilles municipal abattoirs. The adult, on the other hand, occurred in only two of the dogs examined, or 5·4%. Hydatid disease is not uncommon in Man in the South of France: a recent number of the "Archives de Médicine Génerale et Coloniale," published in Marseilles, was almost exclusively devoted to a discussion on Hydatid Disease from the Clinical and Zoological standpoint.

Dipylidium caninum (Linn. 1758) Bailliet 1892.

This common parasite constituted by far the most frequent infection. Twenty-six dogs, or 70·2% of those examined, contained specimens. Many of the infections were intensely heavy, the lower part of the intestine being sometimes nearly blocked for two or three feet by the worms.

Hitherto about a dozen species of *Dipylidium* have been distinguished from dogs and cats. Thus Lewis records six species from the dogs and cats of Aberystwyth. Recently Witenberg (1932), in a full and detailed review of the subfamily *Dipylidiinae,* has shown very convincingly that all these species are synonyms for *D. caninum* with the possible exception of *D. buencaminoi* Tubangui. Too much attention has hitherto been paid to the number of rostellar hooks, which are very easily lost, and to the number of testis lobes which vary from segment to segment.

Mesocestoides lineatus (Goeze, 1782).

Found in one dog only, (2·7%), cohabiting with *D. caninum* and *T. pisiformis*. It has been recorded from Toulouse by Bailliet.

In addition to the thirty-seven dogs from the city of Marseilles which were examined, Professor Joyeux very kindly placed at my disposal some unpublished notes on the post mortem examination of nine dogs from Bastia, Corsica, which gave the following results :—

Out of the nine dogs examined,

Taenia pisiformis	occurred in	3	dogs.
Dipylidium caninum	,,	7	,,
Taenia hydatigena	,,	1	,,
Toxascaris leonina	,,	2	,,
Toxocara canis	,,	1	,,
Ancylostoma caninum	,,	1	,,

The findings here described may be usefully compared with surveys made in other parts of the world.

	Marseilles. Solomon 1933	Aberystwyth. Lewis 1927	Cambridge. Nuttall & Strickland 1908	London. Brown & Stammers 1922	Washington D.C. Sommer 1896	Detroit, Mich. Hall 1917	South Russia. Skrjabin 1924	Philippine Islands. Wharton 1917	Mañaos, Brazil. Gordon & Young 1922
Toxocara canis ...	13·5%	16·3%	70·5%	16·6%	28%	63·5%	25·8%	—	8%
Toxascaris leonina	19·9%	—				—	46·1%	6·7%	6%
Dochmoides stenocephala	13·5%	36·3%	—	—	56%	—	31·4%	—	—
Ancylostoma caninum	—	—	—	—	—	31·1%	7·8%	96·6%	100%
Ancylostoma braziliense	—	—	—	—	—	—	—	—	77%
Trichuris vulpis ...	—	—	—	—	70%	—	—	—	—
Spirocerca sanguinolenta	—	—	—	—	2%	—	23·6%	5·9%	—
Dioctophyme renale	—	—	—	—	2%	2·7%	—	—	—
Haemostrongylus vasorum	—	—	—	—	—	—	1·1%	—	—
Dirofilaria immitis	—	—	—	—	—	—	1·1%	—	4%
Gnathostoma spinigerum	—	—	—	—	—	—	—	6·7%	—
Taenia pisiformis	40·5%	5·1%	16·6%	11·1%	12%	2·7%	1·12%	—	—
Taenia hydatigena	10·8%	13·5%	—	—	2%	2·7%	23·6%	—	—
Taenia multiceps	—	3·4%	—	—	—	—	—	—	—
Echinococcus granulosus	5·4%	5·1%	—	—	—	—	14·6%	—	—
Mesocestoides lineatus	2·7%	—	—	—	—	—	32·5%	—	—
Diphyllobothrium sp.	—	—	—	—	—	—	—	5·9%	—
Dipylidium caninum	70·2%	frequent	58·3%	5·5%	44%	41·8%	77·5%	46·6%	20%
Capillaria plica ...	—	—	—	—	—	—	4·7%	—	—
No. of dogs examined ...	37	59	24	36	50	74	89	118	50

Where the writer quoted has given the percentage of *infected* dogs containing each parasite this figure has been replaced by the percentage of the *total* number of dogs examined, found to be infected.

Trematodes are not included.

Summary.

Post-mortems carried out on 37 dogs in Marseilles showed that all except one carried intestinal Cestode infections, while 15 carried intestinal Nematodes. By contrast to these intestinal infections, all the other

organs examined were remarkable free from parasitosis. The percentage of dogs infected with each parasite is given and, by means of a table, compared with the results of similar investigations in other parts of the world.

Points of interest are the large number of dogs infected with *Taenia pisiformis*; the comparative rarity of hookworm infections (*Dochmoides* in 13·5%; *Ancylostoma caninum* in none); and the presence of *Echinococcus* in between 5% and 6% of the dogs of the town. Though a low figure this is sufficient to constitute a danger to the human inhabitants of the poorer quarters where vegetables and other food might become contaminated by infected dogs.

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A New Blood Fluke from an Indian Tortoise, *Trionyx gangeticus.*

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The hermaphroditic Trematodes from the blood vessels of the turtles are included under the Family Spirorchidae and represent several genera differing from each other in several features, including the general topography of the genital organs. It originally contained only two genera—*Spirorchis* and *Hapalotrema*, differing from each other in the number of suckers, the relative position of the genital glands and the number of testes. Owing to these unique differences between them they were referred by Stunkard (1921) to two subfamilies—Spirorchinae and Hapalotreminae. The work prior to 1923 is summed up by Stunkard (1923) in a brilliant memoir, where, besides describing two new genera, one under each of the two subfamilies, from North American turtles, he has clearly elucidated some important points of systematic nature. Subsequently, the same author has considerably added to our knowledge of the group in several memoirs mentioned in the list of references at the end of the present communication.

The subfamily, Hapalotreminae Stunkard, 1921, contains only two genera, *Hapalotrema* and *Hapalorhynchus*, each with peculiar characters. In the course of our investigations on the entozoa of the local reptiles, we have been able to recover several specimens of the blood flukes from the larger blood vessels and the heart of the mud turtle, *Trionyx gangeticus*, found in the river Gomti, at Lucknow. These specimens, on closer examination, appear to belong to an entirely new genus and are named *Tremarhynchus indicus* n.g., n.sp. These present certain interesting features of morphology that throw light on the affinities of the two already known genera of this subfamily and serve as a connecting link.

Tremarhynchus indicus, n.g., n.sp.

The body of the fluke is elongated, cylindrical, and pointed at either end. The length varies from 3·16 mm. to 3·45 mm., and it has its greatest breadth of ·35–·47 mm., at about the level of the posterior testis. The general surface of the body is smooth and does not bear any spines.

The oral sucker is situated at the extreme anterior end of the body and is extremely protrusible. It is smaller than the ventral sucker and measures ·12 mm. by ·15 mm. The ventral sucker is circular and slightly protrusible. It is situated at about one-third the distance from the anterior end of the body and has a diameter of ·2 mm.

The mouth is in the centre of the oral sucker at the anterior end of the body. The excretory pore lies at the posterior extremity, and the genital pore is on the dorsal side behind the position of the acetabulum.

The mouth leads into an elongated straight tube, the oesophagus, that extends for about two thirds the distance between the two suckers from the anterior end. It has a cuticular inner lining of its wall. The pharynx, as in other blood flukes, is absent, and the oesophagus bifurcates in front of the acetabulum into two intestinal cæca that run backwards to the posterior end, as slender straight tubes of a more or less uniform diameter. Before terminating blindly, at about one-eighth of the body length from the posterior end, the two cæca converge for a small distance.

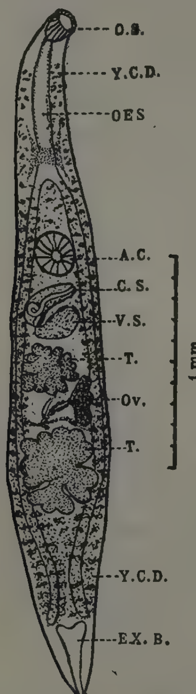
The excretory pore is terminally situated at the posterior end of the body and leads into a large triangular median collecting sac, the excretory bladder. The excretory bladder extends as far forward as the posterior end of the intestinal cæca, where it divides into two narrow lateral ducts one on either side of the median line. The collecting excretory bladder itself is ·2–·3 mm. long and is a very characteristic feature of the group.

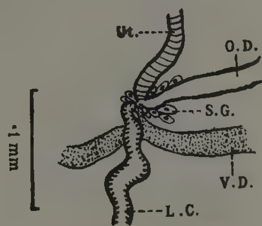
The nervous system is well developed and consists of a distinct œsophageal commissure, situated a little in front of the point of bifurcation of the intestinal cæca, at a distance of ·45–·5 mm. from the anterior end of the body. It appears distinctly X-shaped and runs round the œsophagus. It further sends a pair of nerves towards the anterior end and a pair towards the posterior end of the body.

The female reproductive organs consist of a single ovary situated between the two testes, on the left side of the median line. It is a trilobed organ and leads by a narrow oviduct at about the middle of its right side. The oviduct receives after a short course the common duct of the vitelline glands and thus forms an oötype, at the point of its union. Here Laurer's canal also meets and itself opens on the dorsal surface. The point of union of all these ducts is further marked by the presence of minute unicellular shell glands, shown in Fig. 2 (s.g.).

The vitelline glands are very extensive and irregularly scattered throughout the body. They consist of masses of follicles extending on either side of the median line. In front of the acetabulum and behind the posterior testis they form continuous masses filling up all spaces between the dorsal and ventral walls. In between the acetabulum and the posterior testis they are limited and occupy narrow tracts on either side of the intestinal cæca.

The uterus is very short and arises from the oötype forwards. The eggs have not been observed in any of the specimens so far obtained. The





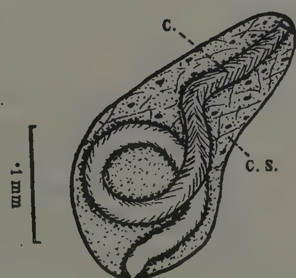


Fig. 1. *Tremarhynchus indicus*, General Anatomy.

Fig. 2. Oötype with various ducts meeting.

Fig. 3. *T. indicus*: Cirrus sac and its contained structures, greatly enlarged. ac. acetabulum; c. Cirrus; c.s. cirrus sac; ex. b. excretory bladder; int. intestinal cæca; l.c. Laurer's canal; od. oviduct; oes. œsophagus; o.s. oral sucker; ov. ovary; s.v. seminal vesicle; s.g. shell glands; t. testes; ut. uterus; v. vitelline glands; v.d. vitelline duct.

absence of the eggs is due to the fact that they are discharged as soon as they are formed. There is not sufficient space for them to remain long within the narrow body of the flukes that are migrating in the blood stream from one part to the other of the body of the host. I have, therefore, nothing to add about the structure of the eggs.

There are two testes situated one in front and the other behind the ovary. The testes are divided into a number of distinct lobes that are deeply cleft, showing a varying number of follicles. The posterior testis is the larger of the two and lobulations are very clearly marked.

There is a large seminal vesicle, more or less oval in outline, that lies transversely across in front of the anterior testis and outside the cirrus sac. It is about ·2 mm. long and leads into an elongated, pearshaped cirrus sac in front. This structure (Fig. 3) is obliquely placed in the body and encloses within it an elongated cirrus. The cirrus, in its proximal portion, is a coiled structure forming as a sort of rudimentary ductus ejaculatorius. The distal part is, however, a straight muscular tube with a slight bend. This is the true cirrus and can be protruded out at the genital pore. Within the cirrus sac there are faint glandular cells that represent the prostate glands.

The genital pore is situated on the dorsal side of the body and is on the left side of the median line.

The distinguishing characters of the genus *Tremarhynchus* may be summed up thus :

" Hermaphroditic blood inhabiting distomes with protrusible suckers ; no cuticular spines ; narrow pointed posterior end ; bicornuate excretory bladder ; testes separated by the ovary and divided into follicles ; seminal vesicle and cirrus anterior to the testes ; genital pore dorsal and sinistral ; vitellaria extensively developed ; pharynx absent."

Host :—*Trionyx gangeticus* (the common mud turtle of Northern India).

Discussion.

The subfamily Hapalotreminae, as originally constituted by Stunkard (1921), contained only the genus *Hapalotrema* Looss, 1899. Later, Stunkard (1923) added another genus *Hapalorhynchus* to it and this was distinguished from *Hapalotrema* in " the absence of the body spines, protrusible oral sucker, simple testes not divided into follicles, absence of cirrus and cirrus sac, presence of large prostate glands, the position of

the seminal vesicle anterior to the testes and the shape of the eggs." To this must now be added a third genus *Tremarhynchus* described in the present communication.

An examination of the various characters of these three genera indicate that the present form is of great systematic importance in so far as it appears to connect the other two genera together. It bears characters in which it resembles the genus *Hapalotrema*, while it has other features that show its affinity with the genus *Hapalorhynchus*. Thus, in the presence of the follicular testes, lobed ovary and the presence of seminal vesicle outside the cirrus, it resembles the genus *Hapalotrema*. In *Hapalotrema*, however, the posterior end of the body is spatulate, the body is armed with spines and the seminal vesicle and the cirrus, as also the genital pore, are situated besides the ovary on its left side between the testes. In these features, therefore, and particularly in the position of the seminal vesicle, cirrus and the genital pore, in front of the testes, the genus *Tremarhynchus* differs remarkably from *Hapalotrema*. In the protrusible nature of the oral sucker, in the absence of the body spines and in the anterior position of the seminal vesicle and the genital pore, the present form resembles the genus *Hapalorhynchus*. But in this latter genus, the testes are not divided into follicles, the prostate glands are large and the cirrus and cirrus sac are absent. Thus, they can readily be distinguished from each other and the new genus *Tremarhynchus*, in these characters at least, can be identified with *Hapalotrema*, from which it has already been shown to be different in certain important features. It would, thus, appear that the new genus presents features common, partly with one and partly with the other, to the two already known genera of the subfamily, Hapalotreminae and is interesting in so far as it forms a connecting link between the two known genera—*Hapalotrema* and *Hapalorhynchus*. The presence of the cirrus and its position along with that of the seminal vesicle, in front of the two testes in the body is, however, unique in the subfamily. All these characters taken together amply justify the creation of our new genus for these flukes.

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The Effect of Heavy Stocking on the Worm Burden under a System of Rotational Grazing.

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THE developments which have taken place during recent years in the scientific management of grassland have resulted in a marked improvement both in the quality and in the quantity of the herbage produced. This has been brought about partly by frequent manuring, particularly with nitrogenous manures, and also by ensuring the close cropping of the grass at frequent intervals.

Increased danger to animals from worm infestation would appear to be an obvious consequence of the heavy stocking which these methods entail. Theoretically, as Taylor (1930) has pointed out, where one sheep on an acre may pick up 10 worms daily, two sheep with the same level of infection would pick up 40 worms daily, the number " increasing as the square of the number of sheep per acre." Other factors such as the effect of close cropping, nutrition and that of grazing with mixed stock undoubtedly influence this risk due to overcrowding but little exact information is so far available on these questions.

It appears probable that under certain circumstances, heavy stocking may not result in an increase in worm infestation but rather in a lowering in the number of worms carried by the animals. Ross and Graham (1933) in their experiments in Australia found that the increased risk of worm infestation when sheep were heavily stocked on improved pasture was more than compensated for by the improved condition of the animals. There was a marked increase both in mutton and wool production in such sheep as compared with a flock on natural pasture. Furthermore, the improved condition of the experimental sheep resulted in their having a greater resistance to worms so that fewer worms were actually harboured by this flock.

It should be pointed out, however, that the stocking capacity of the improved pastures in this experiment ranged from 2½ to 3 sheep per acre. This is considerably below the number which can be carried on improved pastures in this country and it therefore seems possible that a danger zone in worm contamination had not been reached in the Australian experiment at this level of concentration of stock.

That nutritional factors have an important bearing on the worm burden of sheep on pasture has been shown by Fraser and Robertson (1933). These workers found that fewer worms were present in the fourth stomach of lambs, which received concentrated food, on pasture, as compared with the number in a poorly fed group on pasture alone. In this case, however, it might be urged that, owing to extra feeding, the grass requirement of the group receiving extra food would be appreciably less and that the chances of picking up worms would thereby be diminished.

It is clear that much further work is necessary before a true estimate of the effect of heavy stocking on worm infestation can be made and that the consequences of modern grazing practices, as far as worms are concerned, are by no means as self-evident as might be supposed. Certain factors inherent in these practices would appear to have some bearing on the helminthic problems involved and the experiment described below was set up at the Institute of Agricultural Parasitology with a view to obtaining further information on these questions.

Owing to difficulties, chiefly in the setting up of adequate controls, which are met with when field experiments are carried out with living animals, the results obtained cannot be considered conclusive, but it was thought that their publication might prove of interest to others interested in the practical aspects of agricultural helminthology.

The New System of Grassland Management.

It was decided to make observations on animals grazing under what is now commonly called "The New System of Grassland Management." The system aims at increasing the stock-carrying capacity of land by stimulating a rapid growth of young grass. This is attained by frequent applications of quick-acting nitrogenous manures, and by ensuring a suitable sequence of cropping over a series of about seven plots, the grass not being left ungrazed for too long a period to get rank. Each plot is grazed down closely in a period of a week or so and the rotation of the

stock over the plots is so arranged that the first plot is ready for grazing when the last in the series has become bare. As each plot becomes grazed down the animals are moved on to the next and the grazed plot is then harrowed and a dressing of manure applied. In practice it is usual to graze the plots with two groups of animals, the more important stock being put on first and then followed by such animals as store-cattle or horses. It is important to note that it is considered essential in this system to graze each plot down closely and evenly over the whole area during each grazing rotation. A great increase in the concentration of stock per acre has been found possible by this method of rotational grazing and particularly so on pastures which had not been previously well managed. Gardner and others (1931) found an increase of 20 to 30 per cent. of stock possible even on a good old-established pasture which had already reached a high level of productivity.

Description of Experiment.

An old pasture which had carried sheep for a number of years and had been grazed by them during the winter of 1931–32 was chosen for the experiment. The sheep were known to be carrying a mixed infection of helminths and the area used had been fairly heavily contaminated with the droppings of these animals. After applying dressings of phosphates and potash to the whole field early in 1932 a quarter of an acre was fenced off and then divided into six equal plots. An adjoining area equal to three-eighths of an acre (*i.e.*, equal to one and a half times the area occupied by the six experimental plots) was also fenced off but not divided into plots ; this was used as a control plot. The size of the area used as control was based on the assumption that, without extra manurial treatment and rotational grazing, fifty per cent. more pasture would be required to graze the same number of animals as were carried by the six experimental plots.

Since it was desirable to start the experiment with worm-free stock and as sheep of this description were not available it was decided to use two year old goats which had been stall fed from birth and kept practically free from parasites. The number of goats which would be required to graze each plot closely in about seven days could not be ascertained without trial ; this was found after the first week's grazing to be five.

Owing to the wet and cold weather which prevailed during April and May it was not possible to put the animals out on the plots until the 12th of May. During this time the grass on the plots which had received the earliest dressings of Sulphate of Ammonia had grown rather long and it was decided to scythe these plots over lightly, the cut grass being used to feed the selected goats indoors. After the goats were put out the general management of the plots followed, as closely as possible, that adopted under the " New System of Grassland Management."

Except for the first few weeks the plots were grazed fairly closely each time and four rotations were completed by the middle of October.

Technique.

It was hoped to obtain, by a frequent examination of the droppings, some estimate of the worm burden of animals kept under rotational grazing on the experimental plots as compared with that in the animals on the control plot. This was attained by fortnightly counts of the eggs in a gramme of the faeces passed by the goats. These counts were made by shaking up 6·6 gm. of faeces in a flask containing a solution of N/10 Sodium Hydroxide. After obtaining a complete comminution of the faeces by shaking at frequent intervals over a period of not less than three days the contents of the flask were made up to 200 cc. with NaOH.

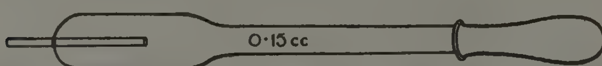

Fig. 1. Diagram of the McDonald pipette used in egg counting technique.

After further shaking, 0·15 cc. of this was drawn up in a pipette and placed on a slide and the number of eggs counted. The average count from three to four samples when multiplied by 200 gave the number of eggs per gramme.

A new type of pipette designed by Mr. W. A. McDonald of the London School of Hygiene and Tropical Medicine was used for taking samples for egg counting. This pipette (Fig. 1) is a modification of the composite pipette as described by Wright (1912) and consists of a glass tube with a length of about 15 cms. and a diameter of about 8 mm. At one end of the tube there is a wide bulb-like expansion into which is sealed a short glass

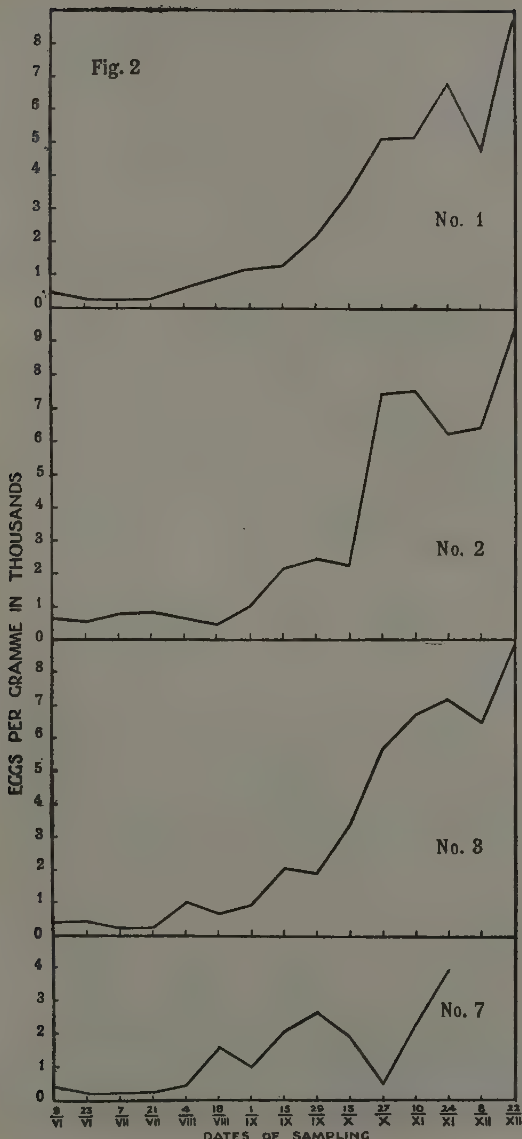

Fig. 2. Graphs shewing rise in egg counts per gramme of faeces from Experimental Goats Nos. 1, 2, 3 and 7 from June 9th to December 22nd.

tube with a diameter of 1½ to 2 mm. and of a length sufficient to hold 0·15 cc. of fluid when full. To the other end of the larger tube is fitted a rubber teat and this is so arranged that the amount of fluid drawn up by it is only slightly in excess of that required to fill the small tube. The excess fluid falls into the bulb which is made large enough to hold a fair quantity of fluid before reaching the level of the inner end of the small tube. This allows for several samples to be taken before it becomes necessary to wash out the pipette. The small tube can be made to project about 1½ to 2 cm. beyond the end of the bulb and when drawing off a sample the pipette can be inserted in the fluid as far as the base of the bulb. This enables samples to be taken from the same level each time.

During the counts those eggs which indicated the type of parasite present were also noted and throughout the period eggs of *Capillaria*, *Nematodirus* and *Trichuris* were frequently met with but were never present in large numbers and showed little tendency to increase. Over a period of three fortnightly counts, *Moniezia* eggs appeared in large numbers in one goat but none were met with afterwards.

Cultures of faeces were also made from time to time and the species, as far as possible, identified by an examination of the infective larvae produced.

Result of Faecal Examinations.

By the 9th of June the egg counts in all the goats had reached a number varying from 100 to 600 per gramme and although from this time onward there was, on the whole, a steady increase, no marked rise occurred until the autumn. While all the experimental goats became uniformly heavily infected a good deal of variation existed in the controls. Goat No. 4 (see Fig. 3) remained throughout at a very low level, while No. 5, on the other hand, reached a level comparable with that found in the experimental goats. In Nos. 6 and 8 in the controls the number varied between these two extremes.

The sharp rise in egg counts which occurred in almost all cases about October is interesting since it coincides with the lowering of the quantity and quality of the herbage at this time of the year. Some loss of condition was also noticed particularly in the experimental goats towards the end of this month. All the plots had become rather bare at this time and the goats were, therefore, given extra food in the form of hay and concentrates. The control goats were fed in the same way although this plot

still showed a fair quantity of rough herbage in places. At this time also the weekly rotations were discontinued and the experimental goats were allowed to roam freely over the six plots.

In reviewing this period from the beginning of the experiment to the end of October, a period which roughly coincides with that during which animals would normally be subjected to rotational grazing, where the

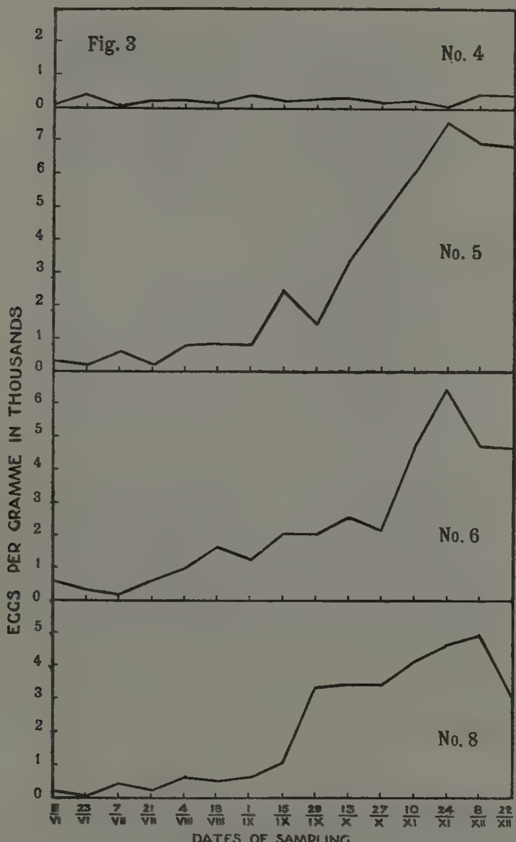

Fig. 3. Graphs shewing rise in egg counts per gramme of faeces from Control Goats Nos. 4, 5, 6 and 8 from June 9th to December 22nd.

" New System " is practised, it can be said that the concentration of goats on the experimental plots was not too great except possibly towards the end of this period. The level of the worm burden had been kept comparatively low during the summer months and did not show any rapid increase until about October. As already stated this rise might be correlated with the lowering of condition in the animals due to lack of herbage and the necessity for closer cropping. On the other hand it might be urged that the contamination of the land had not reached a danger point until the autumn and that this alone would be sufficient to account for the rise in the worm count obtained at this time.

From the beginning of NOVEMBER onwards all the goats were fed with hay and concentrates on the same plots. The experimental goats were, however, no longer subjected to rotational grazing but were allowed to roam over all the six experimental plots.

An interesting feature which began to appear in the experimental goats, from November onwards through the winter, was the condition of the faeces. All the experimental goats passed " mushy " stools while in the case of the controls all, with the exception of Goat No. 5, invariably passed well formed pellets. Fig 4 shows clearly the difference in the condition of the faeces between the two groups of animals.

The result of this condition was that egg counts lost much of their comparative value owing to the varying amount of moisture in a weighed sample of faeces from different goats and although these counts were continued throughout the winter they only served in a general way to indicate the level of the worm burden. Some attempt was made to estimate the moisture content of " mushy " faeces as compared with a sample of formed pellets of the same weight, but it was impossible owing to pressure of other work to carry this out for all samples and was, therefore, abandoned. It is concluded, however, that an egg count based on the weight of dry matter in a sample would probably be the best means of overcoming this difficulty which arises frequently when animals are out on pasture.

Another interesting feature which occurred during the winter period was that all the experimental goats died, whilst only one out of five was lost on the control plot. The first goat (No. 7) died on the 27th of November and although the counts as shown in the graph (Fig. 2) are

Fig. 4. Shewing difference in consistency of faeces between Experimental Goats (Nos. 1, 3 and 7) and Control Goats (Nos. 4, 6 and 8).

(To face page 176.)

rather low the post-mortem examination showed a very heavy infestation with worms. The low counts obtained immediately prior to its death are entirely due to the condition of the faeces which, owing to the amount of moisture it contained, showed a very small number of eggs per gramme. In fact all the goats which died had actually the highest egg counts and their droppings remained consistently softer than it was in those which survived the winter. The only goat (No. 5) which died among the controls had the highest egg counts and was the only one in this group to have " mushy " faeces.

Results of Post-Mortem Examinations.

The post-mortem examination of these goats showed, in varying degree, a congestion of the lungs and enteritis with often a good deal of serous fluid in the body cavities and giving on the whole an appearance of a toxaemic condition. Enormous masses of certain species of worms were present in all cases and it would appear, since death occurred in those with the highest worm burden, that these parasites had been a contributory cause to the death of the animals.

The helminths found in the dead goats contained the following species : *Haemonchus contortus*, *Ostertagia circumcincta*, *O. trifurcata*, *Trichostrongylus axei* (*extenuatus*), *T. vitrinus*, *T. colubriformis*, *T. capricola* (in one goat only), *Capillaria longipes*, *Trichuris ovis* and *Chabertia ovina*.

C. ovina was present in very large numbers in all the goats and together with the Trichostrongyles of the small intestine—particularly *T. vitrinus*—these were throughout the dominant species. *O. circumcincta* was also fairly numerous in some of the animals. *H. contortus*, on the other hand, although found fairly numerous in cultures made from the faeces of the goats during the summer months, was only found in three of the animals and in only one of these was the number (123) appreciable. It would seem that either an immunity had been acquired by the goats to this species or that the winter months are not suitable for its development. In the case of *C. ovina* and the smaller Trichostrongyles, on the other hand, the colder weather had not retarded their development to any appreciable extent since large numbers of larvae in all stages of growth were found at autopsy in all the goats. Under the climatic conditions which generally prevail in this country it is clear, therefore, that helminth larvae can be picked up in considerable numbers even in the winter months. This

applies much more to some species than to others and can probably be accounted for by certain biological differences in the early developmental stages of the parasites.

Discussion.

The somewhat unexpected results obtained during the second period covered by the experiment are difficult to appraise. It would seem that, under the conditions prevailing on the plots during the winter, a point above the danger zone in stock concentration had been reached. The plots, it is true, were rather bare at this period and although the goats were receiving extra food what little grazing was done was sufficient to result in a considerable intake of infective material. The risk of infection would undoubtedly be increased by the necessity for much closer cropping.

The control plot, on the other hand, had not been grazed down so closely and except for one small area a good deal of rank grass remained. In addition, the control plot covered an area which was 50 per cent. greater than that covered by the six experimental plots.

In comparison, the period covering the summer months and early autumn gave sufficient grass to keep the animals in a good thriving condition and, except in October, the grass was not so closely grazed that the larvae, which tend to congregate low down in the herbage, would be picked up in any appreciable numbers.

The results put forward in the following summary can only be considered as provisional in view of the small scale upon which the experiment was carried out and having regard to the fact that the observations cover a period of only one year. Similar work, using larger numbers of animals over a period of several years, is necessary before any recommendations of a practical nature can be made. In fact it is possible that the full effect of over-stocking will not become evident until pastures have been under a system of rotational grazing for many seasons and until the concentration of helminth ova and larvae has reached a very high level. Furthermore, an experiment where young stock is used might well give very different results from those obtained by using older animals, or again, the introduction of " followers " as often carried out in farm practice under the " New System," may have a favourable influence on helminth concentration. This would particularly be the case where the " followers " consisted of a different type of stock from that used as

first grazers on the plots. Such animals in grazing down closely would tend to pick up large numbers of worms.

Finally, this interplay of so varied a group of factors makes the problem of the relationship between heavy stocking and worm infestation a most difficult one and it is further complicated by the incompleteness of our knowledge of the bionomics of many of the common helminth parasites of our domestic animals.

Conclusions.

As a result of the experiments recorded in this paper, the following conclusions may be worthy of consideration.

1. For the period of the year during which the " New System of Grassland Management " is practised, it would appear that certain factors tend to diminish the risk of helminth infestation due to over-stocking.

2. The improved condition of the animals where herbage is plentiful and of good quality may increase their resistance to helminthic infection or at least mask the effect of a heavy worm burden.

3. The abundance of herbage of good quality diminishes the daily grazing period and thus lessens the chances of picking up worms.

4. During the winter months heavy stocking appears to be dangerous in spite of extra feeding with hay and concentrates.

5. Large numbers of larvae may be picked up during winter particularly where grass is scarce and is closely cropped.

6. *Chabertia ovina* and the smaller Trichostrongyles tend to be the commonest species picked up in the winter and appear to have considerable influence on the health of animals. *Haemonchus contortus*, on the other hand, is not common in the same period.

7. In the experiment described in this paper the condition of the droppings of the goats during the winter appeared to vary with the level of the worm burden.

8. All the goats, with one exception which died during the course of the experiment, showed an egg concentration of over 7,000 per gramme of faeces.

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The Bilharzia Complement Fixation Reaction in Goats infected with *Schistosoma mattheei* and *Schistosoma bovis*

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FAILING to obtain a satisfactory antigen from adult bilharzia worms for the serological diagnosis of human intestinal and vesical schistosomiasis in Egypt, Fairley (1919) obtained potent cercarial antigens for this purpose by extracting with absolute alcohol the fresh livers of *Planorbis boissyi* infected with cercariae of *S. mansoni*. This extract, as well as one prepared from *Bullinus contortus* infected with the cercariae of *S. haematobium*, proved equally efficacious in detecting complement fixation antibody in the sera of both human patients and experimentally infected monkeys infested with either parasite. Murray (1920) confirmed these findings in South Africa in patients suffering from *S. haematobium* using the snail vector—*Physopsis africana*—as a source of antigen, and with the view of enhancing its antigenic properties reinforced it with cholesterol.*

Cawston (1921) stated that Murray had found the antigen prepared at Durban from *Physopsis africana* harbouring cercariae of *S. bovis* to be as effective in detecting human infestations with *S. japonicum* and *S. haematobium* as with *S. bovis* in cattle, but no further communication appears to have been made on the subject. Tanabe (1923) using an alcoholic extract of molluscan livers (*Lymnaea palustris*) infected with the cercariae of *Schistosomatium pathlocopticum* obtained positive results in a rat infected with this parasite, as well as with two others harbouring *S. japonicum*.

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*Cholesterol reinforcement is both inadvisable and unnecessary since it leads to pseudo-positive reactions with syphilitic sera without increasing specific antigenic potency.

Fairley (1923) recorded the results of serological diagnosis in a series of goats experimentally infected with *S. spindale*, the antigen being derived from the molluscan intermediary (*Planorbis exustus*). Group reaction was demonstrated for another cattle schistosome, *S. indicum*, as well as for *S. haematobium* in man. Le Bas (1922) confirmed the efficacy of bilharzia cercarial antigen and decided it was protein in nature, but later Fairley (1925) after reinvestigating the whole question adhered to his original view that it was a lipoid or lipoidal complex. Further reports by Fairley (1926) and Fairley & Jasudasin (1930) on the value of the serological test in goats and man followed. Out of 127 goats examined in Bombay 7 gave positive reactions: in these autopsy revealed natural infection with *S. spindale* in four, and with *S. indicum* in two, while the 7th animal gave ++, ± and 0 reactions on three consecutive tests; at autopsy neither worms nor ova were found, but microscopic section of the liver revealed infiltration of Glisson's capsule with mononuclear cells, a finding not uncommon in hepatic schistosomiasis. Most of these animals were heavily infected with intestinal nematodes while many harboured cysticerci in the peritoneal cavity and occasionally a coenurus cyst involved in the brain. No tendency to pseudo-positive reaction was ever observed. In addition over 2,000 tests were made on 150 experimentally infected goats. The conclusion reached from this and other studies was that there was an antigenic complex common to the mammalian schistosome cercariae, and that the test would be found applicable to all types of mammalian schistosomiasis—a prediction supported by the results obtained in the present investigation.

Recent Investigations.

Since 1929 serological observations with this antigen (*S. spindale*) at the Tropical Diseases Hospital, London, has shown it to be a potent antigen for the detection of infestation with *S. mansoni* and *S. japonicum* as well as for *S. haematobium*.

Recently Professor R. T. Leiper suggested investigating the reaction in a series of goats which he had experimentally exposed to infection with cercariae of *S. mattheei*, *S. bovis* and *S. mansoni* during the previous 2 years. The bloods of 18 animals were investigated, the tests being performed without reference to any information regarding exposure of individual animals to infection.

Technique.

The technique employed was similar to that already described in previous publications referred to above, and consisted in the employment of a 1/40 dilution of an alcoholic extract of the livers of *Planorbis exustus* infected with cercariae of *S. spindale*, and testing this against different decomplemented sera (1/5) in the presence of increasing concentration of complement. In the routine tests it was found necessary to employ 4, 6 and 8 minimum haemolytic doses (M.H.D.'s) of complement with English goats, whereas in Indian goats 3, 5 and 7 M.H.D.'s had been found suitable; apparently the sera of normal goats in this country tend to fix more complement than in India; the reason for this is not apparent. In all instances a routine test was performed and known negative goat sera and a known positive human serum from a case of *S. haematobium* fixing 40 M.H.D.'s of complement were included. The reactions were expressed as 0=negative, +=4 M.H.D.'s of complement fixed, ++= 6 M.H.D.'s, and +++= 8 M.H.D.'s. Haemolysis up to approximately 50% in any tube was indicated by the sign ±, and haemolysis in excess of this by the sign +. In addition, in every instance the strongly positive sera (+++) were titrated to an end point, the actual quantity of complement fixed being estimated in terms of M.H.D.'s.

TABLE I.

The Complement Fixation Reaction in Goats Exposed to Infection with *S. mattheei* and Tested against Cercarial Antigen (*S. spindale*).

Number of animal.	Date of 1st exposure to infection.	Ova in faeces.	Duration of disease (days).	Complement Fixation Reaction.		
				Date.	Routine test.	M.H.D.'s fixed.
D7.	11/6/31	+(28/1/33)	695	2/5/33	+++	20
D13.	23/6/31	+(7/7/32)	681	2/5/33	++±	7
D9.	29/6/31	+(7/10/31)	675	2/5/33	0	—
D19.	7/7/31	+(25/6/32)	641	6/4/33	0	—
D2.	7/7/31	+(1/7/32)	667	2/5/33	++±	7
C8.	2/10/31	+(29/1/33)	554	6/4/33	+++	12
C1.	9/10/31	+(15/7/32)	547	6/4/33	+±	5

S. mattheei (Table I).

The sera of 7 goats which had been exposed to infection with cercariae (*S. mattheei*) via the alimentary tract, and in which eggs had been found in the excreta on the dates given in Table I, were tested for the complement fixation reaction. Five of the seven animals were definitely positive, the range of complement fixed varying from 5 to 20 M.H.D.'s. The sera of 2 goats not exposed to infection were negative.

TABLE II.

THE COMPLEMENT FIXATION REACTION IN GOATS EXPOSED TO INFECTION WITH *S. bovis* and *S. mansoni* AND TESTED AGAINST CERCARIAL ANTIGEN (*S. spindale*).

Number of animal.	Date of 1st exposure to infection.	Ova in faeces.	Duration of disease (days)	Complement Fixation Reaction.			Species of cercaria used.
				Date.	Routine test.	M.H.D.'s fixed.	
E4	28/9/32	+3/4/33	192	6/4/33	+++	15	*S. bovis*
E19	25/1/33	+9/4/33	132	6/4/33	+++	12	*S. bovis*
E15.	26/1/33	+9/4/33	131	6/4/33	+++	15	*S. bovis*
E14.	1/2/33	9/4/33 Neg.	126	6/4/33	+++	15	*S. bovis*
D12.	28/1/31	Neg.	—	2/5/33	0	—	*S. mansoni**
C23.	15/12/31	Neg.	—	6/4/33	0	—	*S. mansoni*
D14.	28/1/32	Neg.	—	2/5/33	0	—	*S. mansoni*

*There is no evidence that cercariae of *S. mansoni* produce infection in goats.

S. bovis (Table II.)

The sera of 4 goats first exposed to infection via the alimentary canal with cercariae (*S. bovis*) on the date given in Table II, were examined serologically. Ova had been found in three of these prior to the test. The complement fixation reaction on the other hand was strongly positive in all four animals, 12 to 15 M.H.D.'s of complement being fixed.

The absence of ova from the faeces of E14. by no means implies non-infection for in goats harbouring *S. spindale* faecal examination may occasionally be repeatedly negative for ova, yet autopsy reveal living male and female schistosomes in the portal vein and microscopic examination of colonic and liver tissues, digested with 4% caustic soda, show numbers of ova.

S. mansoni (Table II.)

Three goats which had been exposed to cercariae of *S. mansoni* via the alimentary canal, but in which eggs were absent from the excreta, yielded negative complement fixation reactions. As the antigen used (*S. spindale*) is effective in detecting human infestation with *S. mansoni*, all available evidence indicates that the goat enjoys a natural immunity to this species of parasite. Whether the cercariae actually penetrate the skin and die *in situ* has not yet been determined. Fairley (1927a) found in monkeys, *Macacus sinicus*, enjoying a natural immunity to *S. spindale*, that the schistosomulae developed normally in the portal system for the first fortnight following infection, but rapidly died out in the third week: in these animals the complement fixation reaction became strongly positive following cercariae invasion and remained positive until the end of the third month. No serological observations to date have been made on animals in which all the cercariae die in the peripheral tissues, but on the analogy of the antibody response elicited by subcutaneous injections of cercarial extracts, transient positive reactions would be anticipated provided the cercariae actually penetrated the tissue and were subsequently absorbed, and provided serological tests were carried out at shortly spaced intervals of time.

Discussion.

Apart from its diagnostic value, the extension of the bilharzia complement fixation reaction to *S. mattheei* and *S. bovis* is a matter of considerable immunological interest, for definitive hosts harbouring 7 different species of bilharzia parasites are now known which react in group fashion with the one cercarial antigen—*S. spindale*. Whether the group reaction extends to avian schistosomes remains to be determined, but in view of the additional data reported above its general applicability to mammalian schistosomiasis can no longer be a matter of doubt.

Summary and Conclusions.

1. The sera of 18 goats were investigated for the bilharzia complement fixation reaction, using as antigen alcoholic extract of the livers of snails (*Planorbis exustus*) infected with cercariae of *S. spindale*.

2. The sera of 5 out of 7 goats harbouring *S. mattheei*, and of 4 out of 4 goats exposed to alimentary infection with *S. bovis* yielded positive results, the range of complement fixation varying from 5 to 20 M.H.D.'s.

3. The sera of non-infected goats and of 3 goats exposed to infection with cercariae of *S. mansoni* from which these animals appear naturally immune, yielded negative reactions.

4. The complement fixation reaction with cercarial antigen (*S. spindale*) has now been applied to infestation with 3 human and 4 cattle schistosomes, and its group applicability to mammalian schistosomiasis may be regarded as proven.

Acknowledgments.

I am indebted to Professor R. T. Leiper, F.R.S., for the opportunity of investigating these animals serologically, and for the data related to infection contained in the Tables I and II. The work itself has been made possible by a research grant from the Tropical Diseases Fund, Colonial Office.

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On the Incidence of Stomach Worms in Lambs in the North of Scotland and their Control by Progressive Sectional Grazing

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Introduction.

Recent investigations carried out by the senior writer on the incidence of parasitic helminths in the alimentary tract of sheep in the north of Scotland have demonstrated heavy losses in lambs due to parasitic gastritis. In the autumn of 1932 six severe outbreaks were recorded in the counties of Kincardine, Aberdeen and Moray, but no doubt this number would have been greatly exceeded had it been possible to make a comprehensive survey. Some idea of the severity of the disease may be judged by the fact that several farmers lost from 10–20 per cent. of their lambs.

The usual predisposing cause was observed in each case namely, overstocking attended by failure to move the lambs on to fresh ground when symptoms of parasitic infestation were apparent.

Post-mortem examination of diseased lambs revealed that the lesser stomach worm, *Ostertagia circumcincta* was present in very much larger numbers than any other parasite. *O. trifurcata* sometimes occurred in small numbers but no attempt was made to separate the two species. The table below gives the numbers and species of helminths found in two typical cases of parasitic gastritis.

A further 6 untreated lambs from different areas were autopsied but no record of the actual numbers of worms present was taken. A close examination of the abomasum contents was made, however, and in each case the number of *O. circumcincta* far exceeded that of *H. contortus* or any of the intestinal worms found present. It may, therefore, be concluded that parasitic gastritis in lambs in the north of Scotland is mainly due to *O. circumcincta* and that any control measures directed with a view to checking the disease should be carried out against this parasite.

TABLE I.

SHOWING THE NUMBER AND SPECIES OF HELMINTHS FOUND IN THE INTESTINAL TRACT OF 2 LAMBS SUFFERING FROM PARASITIC GASTRITIS.

Organ.	Species.	Number.	
		Morayshire.	Kincardine-shire.
		Killed 26th Oct. 1932.	Killed 16th Sept., 1932
Abomasum ...	*Ostertagia circumcincta*	16,930	18,110
	Haemonchus contortus	53	76
Small Intestine	*Nematodirus filicollis*	1,238	1,981
	Trichostrongylus vitrinus ...	256	162
	Cooperia curticei	98	354
	Monodontus trigonocephalus ...	7	2
Large Intestine	*Chabertia ovina*	19	26
	Oesophagostomum venulosum ...	8	—
Caecum ...	*Trichuris ovis*	8	12

The fact that in Scotland *H. contortus* occurs in sheep in relatively small numbers compared with *O. circumcincta* is not without interest. Generally speaking in U.S.A. and South Africa the reverse is the case, *H. contortus* being more important from an economic point of view than *O. circumcincta*, although according to a recent report on parasitic diseases in U.S.A. (1932) the lesser stomach worm is stated to be more common in some parts than others, particularly on the West Coast. A similar state of affairs exists in Australia, for according to Seddon and Ross 1929, *H. contortus* is of the greatest importance as the cause of parasitic gastritis in New South Wales and Queensland, but to a less extent in the Southern Australian States where infestation with *O. circumcincta* appears to be more common.

The literature on the control of stomach worms by the administration of anthelmintics is extensive and it is well known that *H. contortus* can be readily controlled by dosing with copper sulphate. Unfortunately *O. circumcincta* is much less susceptible to the action of anthelmintics and no drug which will remove this parasite effectively from the abomasum has as yet been discovered.

Control of stomach worms by a system of folding pastures has long been advocated, and some of the results obtained by this method for *H. contortus* have been highly successful. Such a system of control is based on the nature of the life cycle of the parasite, which has been worked out in detail by Veglia (1916). There does not appear to be any complete account of the life cycle of *O. circumcincta*, but most workers are of the opinion that it follows the same course as *H. contortus* in which the succession of events is as follows.

The eggs laid by the mature females in the abomasum reach the ground in the faeces and the larvae hatch out in about 24 hours. These feed on certain bacteria in the faeces and must pass the next two stages of their development on the ground before they are capable of infecting the sheep. The time taken to reach the infective stage may vary from 3 days to several weeks, depending on the temperature and degree of moisture present.

It can be readily understood that the time taken for the newly hatched larvae to reach the infective stage is the all important factor in regard to the introduction of any system of control by progressive sectional grazing of the pastures. In this connection Taylor (1929) states for both *H. contortus* and *O. circumcincta* that, " although in this country development would rarely be delayed because of excessive dryness, the temperature is usually below that best suited to the larvae and it can only be in our warmest weather that they reach the infective stage in less than 10 days."

Assuming that the larvae of *O. circumcincta* require a period of ten days to become infective it follows that any system of progressive sectional grazing whereby the lambs are shifted on to clean pasture every 10 days should give complete control. Before advising control measures along these lines, it was decided to carry out a preliminary experiment with a view to gaining information on the following points :—

(1) The extent to which stomach worm infestation of lambs can be reduced by a system of progressive sectional grazing.

(2) Whether 10 days is a safe maximum period to allow between successive shifts in reducing infestation from *O. circumcincta.*

(3) The degree to which lambs can become infected when put to graze with their infested dams on clean pasture.

The experiment was undertaken jointly under the North of Scotland College of Agriculture and the Rowett Research Institute, the latter providing all facilities for the investigation.

Plan of the Experiment.

A three acre field of excellent third year's grass which had never carried sheep was fenced into equal portions. Both portions were on a gradual slope and comparable in every respect as regards grazing and drainage.

Six Blackface ewes and their twelve twin Greyface lambs approximately 6 weeks old were put into one half of the field on 25th May, 1932, and allowed to graze over the whole $1\frac{1}{2}$ acres up to the 28th August, 1932.

TABLE II.

Showing the Number of Eggs per Gram of Faeces from Lambs at 6 Weeks old.

P=progressional group.	*N.P.=non-progressional group.*
P.1=85	N.P.1=75
P.2=66	N.P.2=52
P.3=66	N.P.3=81

An equal number of ewes and lambs were allowed to graze only 1/10th of the other half at one time, the ewes and their lambs being changed to clean sections at 10 day intervals. A temporary fence of sheep stakes and wire-netting served to confine the animals.

Both groups of lambs were selected as nearly alike as possible with respect to age, weight and condition. The average weights of the progressional and non-progressional groups were 23·4 lbs. and 23·9 lbs. respectively.

Prior to the commencement of the experiment the ewes and lambs were submitted to faecal examination for the presence of stomach worms. Faecal cultures demonstrated the presence of a moderate to heavy infestation of both *H. contortus* and *O. circumcincta* in all the ewes.

Three lambs from each group were submitted to egg counts by the Stoll method (Table II). The faeces were removed by hand, the method being to insert the small finger into the rectum.

Since it is not possible from an examination of the eggs to determine the species of worms present, faecal cultures were made. Approximately 90 per cent. of the migrating larvae were identified to be *O. circumcincta*, the remaining 10 per cent. being *Nematodirus* and *Trichostrongylus* species. A lamb from the same source and similar in age and weight to those submitted to faecal examination was autopsied and examined for helminths. The abomasum yielded 76 *O. circumcincta* but no *H. contortus*, while the small intestine, colon and caecum contained no helminths. It may be concluded therefore that both groups of lambs commenced the experiment with an initial infection of helminths consisting almost entirely of *O. circumcincta*.

Progress of the Experiment.

Owing to the scarcity of grass at the commencement of the experiment it was found necessary to change the progressional group to the second section after a period of 5 days. Each subsequent section was grazed for an interval of 10 days. Towards the first week of June the grass came away quickly, in fact too quickly, as the rank nature of the grass in the ungrazed sections led to much trampling and wastage of the grass, so much so, that by the end of the 10 day interval the lambs were not finding sufficient for their requirements. This meant that the lambs in the progressional group were getting less food than the non-progressional group and could, therefore, not be expected to put on the same weight. The difficulty could have been met by increasing the grazing area of the progressional group, but since the main object of the experiment was to find out to what extent sectional grazing would lessen the degree of infestation to stomach worms, it was considered inadvisable to take this step. From a nutritional point of view then the non-progressional group had a considerable advantage over the progressional group, as the grass in their plot was plentiful to the end of the experiment.

On the 28th August the lambs were weighed and then removed to a local abattoir for slaughter (Table III). The abomasums were removed and the worms were isolated by the usual process of sedimentation and decantation and counted (Table IV).

TABLE III.

SHOWING THE WEIGHT OF THE LAMBS AT BEGINNING AND THE END OF THE EXPERIMENT.

Progressional Group=P.			*Non-progressional Group*=N.P.		
	Date of Weighing.			Date of Weighing.	
	25th May, 1932	28th Aug., 1932		25th May, 1932	28th Aug., 1932
P.1	21·5	57	N.P.1	24	61
P.2	21	52	N.P.2	23·5	64
P.3	22·5	54	N.P.3	21·5	67
P.4	27	55	N.P.4	21	67
P.5	20	48	N.P.5	25·5	68
P.6	28·5	57	N.P.6	28	73

	P. Group. Lbs.	*N.P. Group.* Lbs.
Average weight of the lambs at the beginning of the experiment	23·4	23·9
Average weight of lambs at the end of the experiment ...	53·8	66·6

TABLE IV.

SHOWING THE TOTAL NUMBERS OF WORMS RECOVERED FROM THE ABOMASUMS OF THE PROGRESSIONAL AND NON-PROGRESSIONAL GROUPS.

Progressional Group=P.			*Non-Progressional Group*=N.P.		
	H. contortus.	*O. circumcincta.*		*H. contortus.*	*O. circumcincta.*
P.1 ...	1	1	N.P.1 ...	452	1,177
P.2 ...	0	36	N.P.2 ...	349	1,132
P.3 ...	1	384	N.P.3 ...	233	927
P.4 ...	2	437	N.P.4 ...	369	1,023
P.5 ...	8	132	N.P.5 ...	86	690
P.6 ...	0	6	N.P.6 ...	61	246

Discussion of Results.

In the case of *H. contortus* the total number of worms recovered from the 6 lambs of the non-progressional group was 1,550, or an average of 258 per lamb. From the progressional group only 12 worms were recovered, giving an average of 2 per lamb. These results show that progressional grazing was efficient to the extent of reducing *H. contortus* infestation 99·2 % compared with the non-progressional group. In other words *H. contortus* can be completely controlled by this system.

The average number of *O. circumcincta* recovered from the non-progressional and progressional groups was 865 and 166 respectively, giving an efficiency of 80·8%. The lower percentage of reduction in the case of *O. circumcincta* would point to the fact that some larvae were able to reach the infective stage in less than 10 days. For all practical purposes, however, 10 days may be considered a safe period for this species, as the few larvae that may develop to the infective stage within that time are not likely to cause much harm.

Statistical analysis of these results kindly undertaken by Dr. Tocher, Aberdeen, indicate that the odds against the results being fortuitous are 700 to 1, and 1,000 to 1, for *H. contortus* and *O. circumcincta* respectively.

The number of worms recovered from the non-progressional group indicate the degree to which lambs may become infested when put to graze on worm-free pasture with their infected dams. Although the numbers are too small to cause serious harm, the evidence shows the advisability of dosing ewes and having them as free of worms as possible before putting them to graze with their lambs on new grass.

Acknowledgments.

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IMPERIAL BUREAU OF AGRICULTURAL PARASITOLOGY

NOTES AND MEMORANDA

No. 10.

The Helminth Parasites of Marsupials

ALTHOUGH some marsupials are insectivorous or carnivorous in diet many others are vegetarian in diet and frequently cause appreciable damage to pastures and crops and therefore are of considerable economic importance. Recently, during the course of searching the literature for references to helminth infections in marsupials it was observed that, in certain cases, hosts other than marsupials were involved and in some instances were of economic importance in that they were domesticated animals. Whether or not marsupials assist in the spread of parasitic infection is somewhat inadequately dealt with in the literature but one cannot lose sight of their acting as reservoir hosts.

DISTRIBUTION AND NOMENCLATURE OF MARSUPIALS.

The term " Marsupials " is here used as indicative of the Mammalian Order Marsupialia (*Metatheria* or *Didelphia*) of which the principal characteristic is the possession by most forms of a sac or pouch (*marsupium*), which is supported by two epipubic bones, enclosing the teats of the mammary glands and receiving the helpless young at birth. The marsupials are in the main confined to the Australian and Austro-Malayan regions, but two families, one of which is the Didelphiidae, are found in the neotropical region to which they are peculiar. The majority of the forms are found in Australia although there are a number which occur in the eastern Austro-Malayan Islands, including the Celebes.

The group contains about 180 species. In the list appended to this memorandum are included 21 genera and the more detailed distribution of each species is given on pp. (36) to (52). An examination of this host-list will show that the nomenclature of marsupials is somewhat confusing even although only those synonyms that occur frequently in helminthological literature are cited. For the purposes of this memorandum Flower's classification has been followed, as given in the " List of the vertebrated animals exhibited in the gardens of the Zoological Society of London, 1828-1927." Vol. I, " Mammals," London, 1929, ix + 419 pp. In conjunction with this, reference has frequently been made to Oldfield Thomas' " Catalogue of the Marsupialia and Monotremata in the Collection of the British Museum (Natural History)." London, 1888, xiii + 401 pp.

The recognized scientific names and their principal synonyms as well as the common names of the 52 species considered in this memorandum are combined in one alphabetical arrangement in the host-list, but for the convenience of readers more conversant with the popular names these are listed in the following Table :—

Popular Names of Marsupials.

Agile Wallaby (*Macropus agilis*).
American Opossum (*Didelphis marsupialis*).
Antilopine Kangaroo (*Macropus antilopinus*).
Aru Island Wallaby (*Macropus brunii*).
Australian Naked-nosed Wombat (same as Mitchell's Wombat).
Azara's Opossum (*Didelphis marsupialis azarae*).
Bennett's Tree-Kangaroo (*Dendrolagus bennettianus*).
Bennett's Wallaby (*Macropus ruficollis bennetti*).
Black Cuscus (*Phalanger ursinus*).
Black-striped Wallaby (*Macropus dorsalis*).
Black-tailed Wallaby (*Macropus ualabatus*).
Black Wallaby (same as Black-tailed Wallaby).
Bridled Nail-tailed Wallaby (*Onychogalea frenata*).
Brush-tailed Pouched-Mouse (*Phascogale penicillata*).
Brush-tailed Rock-Wallaby (*Petrogale penicillata*).
Chilian Opossum (*Marmosa elegans*).
Common Three-striped Opossum (*Didelphis americana*).
Crab-eating Opossum (*Didelphis marsupialis karkinophaga*).
Dama Wallaby (*Macropus eugenii*).
De Bruyn's Wallaby (same as Aru Island Wallaby).
Derbian Wallaby (same as Dama Wallaby).
Eugene Island Wallaby (same as Dama Wallaby).
Fawn Nail-tailed Wallaby (*Onychogalea unguifera*).
Flinders Island Wombat (*Phascolomis ursinus*).
Forester (same as Great Grey Kangaroo).
Four-spotted Opossum (same as Quica Opossum).
Gamba Opossum (same as Pink-tip-eared Opossum).
Great Grey Kangaroo (*Macropus giganteus*).

Grizzled Grey Tree-Kangaroo (*Dendrolagus inustus*).
Herbert-River Phalanger (*Pseudochirus herbertensis*).
Koala (*Phascolarctos cinereus*).
Long-eared Opossum (same as Silver-Grey Opossum).
Long-nosed Bandicoot (*Perameles nasuta*).
Marsupial Wolf (same as Tasmanian Wolf).
Merian's Opossum (same as Murine Opossum).
Mitchell's Wombat (*Phascolomis mitchelli*).
Murine Opossum (*Marmosa murina*).
Native Cat (*Dasyurus* sp.).
Native Bear (same as Koala).
North-Australian Bandicoot (*Perameles macrura*).
Northern Wallaby (scientific name not traced).
Opossum, includes *Didelphis*, *Marmosa*, *Metachirus*, *Philander*, *Pseudochirus* and *Trichosurus*.
Pademelon Wallaby (*Marcopus thetidis*).
Philander (same as Aru Island Wallaby).
Philander Opossum (*Philander philander*).
Pink-tip-eared Opossum (*Didelphis marsupialis aurita*).
Quenda (same as Short-nosed Bandicoot).
Quica Opossum (*Metachirus opossum*).
Rat-tailed Opossum (*Metachirus nudicaudatus*).
Red Kangaroo (*Macropus rufus*).
Red-necked Wallaby (*Macropus ruficollis*).
Red-sided Opossum (*Didelphis brevicaudata*).
Short-eared Opossum or Phalanger (*Trichosurus caninus*).
Short-nosed Bandicoot (*Isoodon obesula*).
Silver-Grey Opossum (*Trichosurus vulpecula*).
Sombre Wallaby (*Macropus browni*).
Spectacled Hare-Wallaby (*Lagorchestes conspicillatus*).
Striped-faced Opossum (same as Azara's Opossum).
Swamp Wallaby (same as Black-tailed Wallaby).
Tammar (same as Dama Wallaby).
Tasmanian Devil (*Sarcophilus harrisii*).
Tasmanian Wolf (*Thylacinus cynocephalus*).
Thick-tailed Opossum (*Metachirus crassicaudatus*).
Thigh-striped Wallaby (same as Pademelon Wallaby).
Tiny Opossum (same as Murine Opossum).
Virginian Opossum (*Didelphis marsupialis virginiana*).
Viverrine Native-Cat (*Dasyurus viverrinus*).
Wallaroo (*Macropus robustus*).
Wambenger (same as Brush-tailed Pouched-Mouse).
Water Opossum (*Chironectes minimus*).
Wied's Opossum (same as Pink-tip-eared Opossum).
Woodward's Wallaroo (*Macropus robustus woodwardi*).
Woolly Opossum (*Philander lanigera*).
Zebra Wolf (same as Tasmanian Wolf).

It was not found possible to avoid the use of the somewhat cumbersome trinomial nomenclature as, in the case of *Didelphis marsupialis*, a number of subspecies have been identified, some of which enter into this list. This also applies to certain subspecies of the genus *Macropus*.

The following names of marsupials have also been referred to in the helminthological literature but no information is obtainable as to the

real species for which they stand : *Didelphis bistriata*, *Didelphis goagnia*, *Didelphis quoaiquiqua* and Northern Wallaby. In the case of *D. bistriata* there is the possibility that it was intended for *D. americana* (synonym *Didelphys tristriatus*). While such popular names as Three-striped Opossum and One-striped Opossum occur there is no mention made, however, of a Two-striped Opossum. Similarly the popular name of Northern Wallaby could not be linked up with any recognized scientific name. The only indication given was that it pertained to a species of *Halmaturus* which is synonymous with *Macropus*.

On certain occasions some authors made use of somewhat vague popular names in their records of parasitic infections and beyond stating that the host was " Kangaroo sp.", " Phalanger," or " Wallaby " no further details were given. To attempt to give a scientific name for any of these bare popular names is out of the question as can be observed in the case of " Wallaby " which includes the genera *Dorcopsis*, *Lagorchestes*, *Macropus*, *Onychogalea* and *Petrogale*. Where the host was not specified a note to this effect has been inserted in the Host-list.

Throughout the literature the names *Didelphis* and *Didelphys* occur frequently but the former spelling has been adhered to in this memorandum as being that generally recognized in modern usage and *Didelphys* has been treated as a synonym.

Economic Importance of Marsupials.

In their mode of nourishment and in their habits, marsupials differ much amongst themselves. Some are purely herbivorous such as the Kangaroos and Wallabies ; others are carnivorous as exemplified by the Dasyures and Thylacines ; but most of them are omnivorous. The Kangaroos and Wallabies are adapted for a terrestrial life and are rarely arboreal and frequent open or sparsely wooded country where they feed upon heather, grass and the young shoots of shrubs and bushes. The animals do much damage to young corn and other crops and frequently destroy pasture by biting it well down to the roots with their very efficient front teeth.

The " Native Cats " or Dasyures are among the best known of the marsupials as they render themselves notorious and obnoxious to settlers and farmers by their serious depredations in poultry-yards and similar

places as well as living in trees and feeding largely upon birds and their eggs.

The Tasmanian Wolf, now restricted to Tasmania, has been more or less driven to the mountains, owing to its former destructiveness to sheep, and is very rare. Its close neighbour, the Tasmanian Devil, is also restricted to Tasmania and is more destructive than the Wolf through killing sheep and fowls, apparently for the mere pleasure of slaughter long after its appetite has been sated.

Amongst the Bandicoots several species occur commonly and are widely distributed. They are well known to crop growers on account of the damage done by them in gardens and cultivated fields. They are fossorial in habit and their food consists of roots, bulbs, berries, fallen fruit and other vegetable substances, in addition to insects and earth worms.

The Brush-tailed Pouched-Mouse is noted as being very destructive to birds.

The large species of the typical opossums, restricted to America, are very destructive to poultry although the smaller forms subsist mainly or exclusively on insects. The Common or Virginian Opossum, although well known as a poultry-thief, is much esteemed for food by the American negroes of the Southern United States who are particularly partial to its flesh. It is also valued on account of its fur.

The Crab-eating Opossum of South America shows a considerable degree of adaptability and besides raiding hen-roosts and orchards, may at times enter towns where it forages for scraps of food.

The Common Phalanger or Australian Vulpine Opossum is found in both Australia and Tasmania. The Tasmanian variety, however, is the larger of the two and is now of considerable value on account of the deep rich brown fur it yields.

On the whole, while considering the large number of species represented in the Order, comparatively few are of very real economic significance.

Helminth Parasites of Marsupials.

The subjoined parasite-list of marsupials is as complete as present information allows. The parasites are listed under the four main Classes : flukes or Trematoda, tapeworms or Cestoda, roundworms or Nematoda

and thorny-headed worms or Acanthocephala. The accepted scientific name of the parasite together with the author and date are to be found on the first line. No synonyms have been inserted as, in many instances, so many are involved that it would be inconvenient to include them all in the list. A striking example of this is in the case of the cestode form *Echinococcus granulosus* which has over 100 recognized synonyms. Below the name of each parasite will be found a list of marsupials from which it has been recorded, the location in the body of the host as mentioned in the literature and the locality from which the infestation was reported. It will be observed by a glance at the list that locality reports in many cases do not coincide with the habitat of the host for the simple reason that, at times, an author has described or mentioned a parasite as occurring in a marsupial which was present in a living collection such as the Gardens of the Zoological Society of London. In a few cases also helminthological specimens have been described from material preserved in collections in museums and about which only data on the collection label was available. In a few instances no information was found regarding the location or locality of the parasites.

Under each parasite name appears one or more abbreviated references to the record of this form in the hosts from which it has been reported. The full references are arranged alphabetically at the end of this memorandum. Many records of parasitism were traced, in the first instance, in text-books or other abbreviated host-lists and, wherever possible, the original record has been found and checked. On a few of the species some comment is desirable and such comments will be found immediately following the list.

Helminth Parasites of Marsupials, with References.

TREMATODA.

Alaria sp. Cameron, 1931.

Host—Sarcophilus harrisii.
Location—Intestine.
Locality—Scottish Zool. Park, Edinburgh.
Reference—Cameron, 1931, p. 153.

ECHINOSTOMUM sp. Dikmans, 1931.

Host—Didelphis marsupialis virginiana.

Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.

Reference—Dikmans, 1931, p. 3.

FASCIOLA HEPATICA Linnaeus, 1758.

Hosts—Kangaroo (unspecified).
Macropus giganteus.
Macropus ruficollis.
Wallaby (unspecified).

Location—Bile ducts.

Locality—Queensland ; New South Wales ; Australia.

References—Johnston, 1909, pp. 516, 518.
Johnston, 1911A, p. 48.
Linstow, 1878, p. 63.

HARMOSTOMUM DASYURI Johnston, 1913.

Host—Dasyurus viverrinus.

Location—Intestine.

Locality—Hunter's Hill, vicinity of Sydney, New South Wales.

References—Johnston, S. J., 1913, p. 727.
Travassos, 1928, p. 327.
Witenberg, 1925, p. 191.

HARMOSTOMUM OPISTHOTRIAS (Lutz, 1895) Braun, 1899.

Hosts—Didelphis marsupialis aurita.
Didelphis marsupialis virginiana.

Location—Small intestine.

Locality—Houston, Texas, U.S.A.
São Paulo, Brazil.

References—Braun, 1899, p. 492 ; 1900A, p. 12 ; 1901, p. 338.
Chandler, 1932, p. 4.
Lutz, 1895, p. 181.
Travassos, 1928, p. 327.
Viana, 1924, p. 137.

HARMOSTOMUM OPISTHOTRIAS var. VIRGINIANA Dickerson, 1930.

Host—Didelphis marsupialis virginiana.

Locality—North America.
Reference—Dickerson, 1930, p. 37.

HARMOSTOMUM SIMILE Johnston, 1913.
Host—Isoodon obesula.
Location—Intestine.
Locality—Vicinity of Sydney, New South Wales.
References—Johnston, S. J., 1913, p. 731.
Travassos, 1928, p. 327.
Witenberg, 1925, p. 191.

HARMOSTOMUM sp. Dikmans, 1931.
Host—Didelphis marsupialis virginiana.
Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.
Reference—Dikmans, 1931, p. 3.

HEMISTOMUM PEDATUM Diesing, 1850.
Hosts—Didelphis marsupialis aurita.
Didelphis marsupialis karkinophaga.
Metachirus nudicaudatus.
Location—Small intestine.
Locality—Brazil.
References—Brandes, 1888, p. 61.
Diesing, 1850, p. 309; 1855, p. 61.
Linstow, 1878, p. 64.
Viana, 1924, p. 140.

LEVINSENIELLA JÄGERSKIOLDI Travassos, 1920.
Host—Didelphis marsupialis aurita.
Location—Intestine.
Locality—Manguinhos (Rio), Brazil.
References—Travassos, 1920, p. 87.
Viana, 1924, p. 127.

MEHLISIA ACUMINATA Johnston, 1913.
Host—Dasyurus viverrinus.
Location—Intestine.
Locality—New South Wales.
Reference—Johnston, S. J., 1913, p. 733.

NEODIPLOSTOMUM LUCIDUM LaRue & Bosma, 1927.

Host—Didelphis marsupialis virginiana.

Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.

References—Dikmans, 1931, p. 3.
LaRue & Bosma, 1927, p. 124.

PLAGIORCHIS DIDELPHIDIS (Parona, 1896).

Host—Didelphis marsupialis azarae.

Locality—Paraguay.

References—Luehe, 1899, p. 532.
Parona, 1896, p. 164.
Stossich, 1904, p. 2.

PROALARIA VARIABILIS Chandler, 1932.

Host—Didelphis marsupialis virginiana.

Location—Small intestine.

Locality—Houston, Texas, U.S.A.

Reference—Chandler, 1932, p. 1.

RHOPALIAS BACULIFER Braun, 1900.

Host—Chironectes minimus.

Location—Intestine.

Locality—Brazil.

References—Braun, 1900B, p. 28; 1901, p. 325.
Viana, 1924, p. 99.

RHOPALIAS CORONATUS (Rudolphi, 1819).

Hosts—Chironectes minimus.
Didelphis marsupialis aurita.
Didelphis marsupialis karkinophaga.
Didelphis marsupialis virginiana.
Metachirus nudicaudatus.
Metachirus opossum.

Location—Intestine.

Locality—Brazil.

References—Braun, 1893, p. 911; 1901, p. 320.
Cobbold, 1879, p. 432.
Diesing, 1850, p. 400.

Dujardin, 1845, p. 425.
Linstow, 1878, p. 64.
Rudolphi, 1819, p. 686.
Stiles & Hassall, 1898, p. 93.
Stossich, 1892, p. 30.
Viana, 1924, p. 109.

RHOPALIAS HORRIDUS (Diesing, 1850).
Hosts—Didelphis marsupialis aurita.
Metachirus nudicaudatus.
Metachirus opossum.
Philander philander.
Location—Stomach, small intestine.
Locality—Angra dos Reis [Est. do Rio], Brazil.
References—Braun, 1893, p. 911 ; 1901, p. 319.
Cobbold, 1879, p. 432.
Diesing, 1850, p. 400.
Linstow, 1878, p. 65.
Stiles & Hassall, 1898, p. 93.

RHOPALIAS MACRACANTHUS Chandler, 1932.
Host—Didelphis marsupialis virginiana.
Location—Small intestine.
Locality—Houston, Texas, U.S.A.
Reference—Chandler, 1932, p. 5.

RHOPALIAS sp. Dikmans, 1931.
Host—Didelphis marsupialis virginiana.
Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.
Reference—Dikmans, 1931, p. 3.

CESTODA.

ANOPLOTAENIA DASYURI Beddard, 1911.
Host—Sarcophilus harrisii.
Location—Zool. Gdns., London.

References—Baer, 1925, p. 2.
Beddard, 1911, p. 1003.
Cameron, 1931, p. 153.
Meggitt, 1924, p. 43.
Ortlepp, 1922, p. 611.

BANCROFTIELLA TENUIS Johnston, 1911.
Host—Macropus ualabatus.
Location—Intestine.
Locality—Victoria, Australia.
References—Johnston, 1911A, p. 50; 1911B.
Meggitt, 1924, p. 57.
Ortlepp, 1922, p. 610.

BERTIELLA ABERRATA Nybelin, 1917.
Host—Pseudochirus herbertensis.
References—Meggitt, 1924, p. 23.
Nybelin, 1917, p. 21.
Ortlepp, 1922, p. 611.

BERTIELLA EDULIS (Zschokke, 1898).
Host—Phalanger ursinus.
Locality—Australia and vicinity; Celebes.
References—Bourquin, 1905, pp. 418, 494, 503.
Douthitt, 1915, p. 418.
Johnston, 1909, p. 520.
Meggitt, 1924, p. 23.
Ortlepp, 1922, p. 610.
Zschokke, 1898A, p. 477; 1899, p. 405; 1904B, p. 60; 1907, p. 264.

BERTIELLA OBESA (Zschokke, 1898).
Host—Phascolarctos cinereus.
Locality—Queensland; New South Wales; Australia.
References—Bourquin, 1905, pp. 418, 495, 503.
Cobbold, 1879, p. 432.
Douthitt, 1915, p. 418.
Johnston, 1909, p. 520.
Meggitt, 1924, p. 23.

Nybelin, 1917, p. 34.
Ortlepp, 1922, p. 611.
Zschokke, 1896, p. 481 ; 1898A, p. 477 ; 1898B, p. 368 ; 1899, p. 422 ; 1904B, p. 60 ; 1907, p. 264.

BERTIELLA PELLUCIDA Nybelin, 1917.
Host—Pseudochirus lemuroides.
References—Meggitt, 1924, p. 24.
Nybelin, 1917, p. 18.
Ortlepp, 1922, p. 610.

BERTIELLA PSEUDOCHIRI Nybelin, 1917.
Host—Pseudochirus herbertensis.
References—Meggitt, 1924, p. 24.
Nybelin, 1917, p. 20.
Ortlepp, 1922, p. 611.

BERTIELLA RIGIDA (Janicki, 1905).
Host—Opossum (Phalanger [*sens. lat.*] sp.).
Locality—Tawarin, New Guinea.
References—Douthitt, 1915, p. 417.
Janicki, 1905, p. 127 ; 1906A, p. 528 ; 1906B, p. 181.
Johnston, 1909, p. 520.
Meggitt, 1924, p. 24.
Ortlepp, 1922, p. 611.
Zschokke, 1907, p. 264.

BERTIELLA SARASINORUM (Zschokke, 1898).
Host—Phalanger ursinus.
References—Bourquin, 1905, pp. 418, 494, 503.
Douthitt, 1915, p. 418.
Fuhrmann, 1899, p. 85.
Johnston, 1909, p. 520.
Meggitt, 1924, p. 24.
Ortlepp, 1922, p. 610.
Zschokke, 1898A, p. 477 ; 1899, p. 404 ; 1904B, p. 60 ; 1907, p. 264.

BERTIELLA UNDULATA Nybelin, 1917.
Host—Pseudochirus lemuroides.

References—Meggitt, 1924, p. 25.
Nybelin, 1917, p. 7.
Ortlepp, 1922, p. 610.

BOTHRIOCEPHALUS DIDELPHIDIS Ariola, 1900.
Hosts—Didelphis marsupialis.
Didelphis marsupialis azarae.
Location—Intestine.
Locality—São Paulo ; Brazil.
References—Ariola, 1900, p. 418.
Fuhrmann, 1902, p. 445.
Janicki, 1906A, p. 520.
Meggitt, 1924, p. 122.
Ortlepp, 1922, p. 611.
Parona, 1901, p. 1.

BOTHRIOCEPHALUS MARGINATUS Krefft, 1871.
Host—Macropus sp.
Locality—Queensland ; Australia.
References—Ariola, 1900, p. 453.
Cobbold, 1879, p. 432.
Johnston, 1909, p. 518.
Krefft, 1871, p. 227.
Ortlepp, 1922, p. 610.

BOTHRIOCEPHALUS sp. Janicki, 1904.
Hosts—Didelphis goagnia.
Didelphis sp.
Location—Larval form in musculature and connective tissue.
Locality—Berlin Museum and Santa Cruz.
References—Janicki, 1904B, p. 771 ; 1906A, p. 516.
Meggitt, 1924, p. 146.

CITTOTAENIA VILLOSA Lewis, 1914.
Host—Lagorchestes conspicillatus.
Location—Stomach, intestine.
Locality—Hermite Island, Monte Bello Islands.
References—Lewis, 1914, p. 427.
Meggitt, 1924, p. 27.
Ortlepp, 1922, p. 610.

DASYUROTAENIA ROBUSTAE Beddard, 1912.

Host—Sarcophilus harrisii.

Locality—Zool. Gdns., London.

References—Baer, 1925, p. 9.
Beddard, 1912, p. 677; 1915, p. 187.
Meggitt, 1924, p. 27.
Ortlepp, 1922, p. 611.

DITHYRIDIUM (PIESTOCYSTIS) CYNOCEPHALI Ransom, 1907.

Host—Thylacinus cynocephalus.

Location—Larval form in heart muscles. [Adult stage unknown.]

Locality—Zool. Gdns., Washington, U.S.A.

References—Johnston, 1909, p. 521.
Meggitt, 1924, p. 147.
Ortlepp, 1922, p. 611.
Ransom, 1907, p. 31.

ECHINOCOCCUS GRANULOSUS (Batsch, 1786) Rudolphi, 1805.

Hosts—Macropus dorsalis.
Macropus eugenii.
Macropus giganteus.
Macropus robustus.
Macropus thetidis.
Macropus ualabatus.

Location—Larval form, Hydatid, in lungs and liver. [Adult in canines.]

Locality—Queensland; New South Wales; Australia.

References—Cobb, 1905, p. 630.
Hall, 1919, p. 58.
Johnston, 1909, p. 516; 1911A, p. 47.
Linstow, 1878, p. 64.
Meggitt, 1924, p. 150.
Neumann, 1905, p. 431.
Neveu-Lemaire, 1912, p. 482.
Ortlepp, 1922, p. 610.
Pagenstecher, 1871.
Railliet, 1893, p. 260.
Stiles, 1906, p. 76.

HEPATOTAENIA DIAPHANA (Zschokke, 1907) Nybelin, 1917.

Host—Phascolomis ursinus.

Location—Liver.

Locality—Tasmania? ; Islands of Bass Strait ; Australia.

References—Douthitt, 1915, p. 399.
Johnston, 1911A, p. 49.
Meggitt, 1924, p. 27.
Nybelin, 1917, p. 29.
Ortlepp, 1922, p. 611.
Zschokke, 1907, p. 261.

HEPATOTAENIA FELLICOLA Nybelin, 1917.

Host—Macropus agilis.

Location—Gall bladder.

References—Meggitt, 1924, p. 28.
Nybelin, 1917, p. 28.
Ortlepp, 1922, p. 610.

HEPATOTAENIA FESTIVA (Rudolphi, 1819) Nybelin, 1917.

Hosts—Macropus eugenii.
Macropus giganteus.
Macropus robustus.
Onychogalea unguifera.

Location—Gall bladder and bile ducts.

Locality—New South Wales and Queensland ; Australia.

References—Blanchard, 1891, p. 444.
Cobbold, 1879, p. 432.
Diesing, 1850, p. 500.
Douthitt, 1915, p. 423.
Dujardin, 1845, p. 593.
Janicki, 1906A, p. 527.
Johnston, 1909, p. 516.
Linstow, 1878, p. 63.
Meggitt, 1924, p. 28.
Nybelin, 1917, p. 25.
Ortlepp, 1922, p. 610.
Rudolphi, 1819, pp. 146, 490.
Stiles & Hassall, 1893, p. 53.
Zschokke, 1896, p. 481 ; 1904B, p. 60 ; 1907, p. 264.

HYMENOLEPIS PERAMELIDARUM Nybelin, 1917.

Host—Perameles macrura.

References—Meggitt, 1924, p. 73.
Nybelin, 1917, p. 34.
Ortlepp, 1922, p. 611.

LINSTOWIA BRASILIENSIS Janicki, 1904.

Hosts—Didelphis americana.
Didelphis bistriata.

Locality—Brazil; South America.

References—Bourquin, 1905, p. 502.
Douthitt, 1915, p. 426.
Janicki, 1904B, p. 770; 1906A, p. 507.
Meggitt, 1924, p. 41.
Ortlepp, 1922, p. 612.
Zschokke, 1904A, p. 291; 1904B, p. 60.

LINSTOWIA ECHIDNAE (Thompson, 1893) Zschokke, 1899.

Host—Isoodon obesula.

References—Bourquin, 1905, pp. 418, 502.
Meggitt, 1924, p. 41.
Ortlepp, 1922, p. 611.

LINSTOWIA JHERINGI Zschokke, 1904.

Host—Didelphis americana.

Locality—South America.

References—Bourquin, 1905, p. 502.
Douthitt, 1915, p. 426.
Janicki, 1906A, p. 512.
Meggitt, 1924, p. 41.
Ortlepp, 1922, p. 612.
Zschokke, 1904A, p. 291; 1904B, p. 60.

LINSTOWIA SEMONI (Zschokke, 1896) Zschokke, 1899.

Hosts—Isoodon obesula.
Perameles nasuta.

Locality—Queensland; Sydney, New South Wales; Zool. Gdns., St. Petersburg.

References—Bourquin, 1905, pp. 418, 502.
Douthitt, 1915, p. 426.
Janicki, 1906A, pp. 507, 512.
Johnston, 1909, p. 521 ; 1911A, p. 50.
Linstow, 1903, p. 282.
Meggitt, 1924, p. 41.
Nybelin, 1917, p. 34.
Ortlepp, 1922, p. 611.
Shipley, 1902, p. 608.
Zschokke, 1896, p. 481 ; 1898B, p. 364 ; 1899, p. 441 ; 1904B, p. 60 ; 1907, p. 264.

LINSTOWIA SEMONI var. ACANTHOCIRROSA Nybelin, 1917.
Host—Perameles macrura.
References—Meggitt, 1924, p. 42.
Nybelin, 1917, p. 33.
Ortlepp, 1922, p. 611.

MESOCESTOIDES sp. Dikmans, 1931.
Host—Didelphis marsupialis virginiana.
Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.
Reference—Dikmans, 1931, p. 3.

MONIEZIA BIPAPILLOSA (Leidy, 1875).
Hosts—Phascolomis mitchelli.
Phascolomis sp.
Locality—Zool. Gdns., Philadelphia ; New South Wales, Australia.
References—Cobbold, 1879, p. 432.
Johnston, 1909, p. 519.
Leidy, 1875A, p. 14 ; 1904, p. 136.
Meggitt, 1924, p. 29.
Ortlepp, 1922, p. 611.

OOCHORISTICA BIVITTATA Janicki, 1904.
Hosts—Marmosa elegans.
Marmosa murina.
Location—Intestine.
Locality—Vienna Museum.

References—Janicki, 1904B, p. 772; 1906A, p. 515.
Meggitt, 1924, p. 43.
Ortlepp, 1922, p. 611.
Zschokke, 1904A, p. 219; 1904B, p. 60; 1905, p. 61.

OOCHORISTICA DIDELPHIDIS (Rudolphi, 1819) Zschokke, 1904.
Hosts—Marmosa elegans.
Marmosa murina.
Location—Intestine.
Locality—Vienna Museum
References—Janicki, 1904B, p. 772; 1906A, p. 513.
Linstow, 1878, p. 64.
Meggitt, 1924, p. 43.
Ortlepp, 1922, p. 611.
Rudolphi, 1819, p. 170.
Zschokke, 1904B, p. 60; 1905, p. 61.

OOCHORISTICA MARMOSAE Beddard, 1914.
Host—Marmosa elegans.
References—Beddard, 1914, p. 269.
Meggitt, 1924, p. 43.
Ortlepp, 1922, p. 611.

OOCHORISTICA MURINA Zschokke, 1904.
Hosts—Marmosa elegans.
Marmosa murina.
Locality—South America.
References—Meggitt, 1924, p. 44.
Ortlepp, 1922, p. 611.
Zschokke, 1904A, p. 219.

PARABERTIELLA CAMPANULATA Nybelin, 1917.
Host—Pseudochirus lemuroides.
References—Meggitt, 1924, p. 33.
Nybelin, 1917, p. 23.
Ortlepp, 1922, p. 610.

PROGAMOTAENIA BANCROFTI (Johnston, 1913) Nybelin, 1917
Hosts—Onychogalea frenata.
Onychogalea unguifera.

References—Johnston, T. H., 1913, p. 68.
Meggitt, 1924, p. 34.
Nybelin, 1917, p. 30.
Ortlepp, 1922, p. 610.

PROGAMOTAENIA LAGORCHESTIS (Lewis, 1914) Nybelin, 1917.
Host—Lagorchestes conspicillatus.
Location—Stomach, intestine.
Locality—Hermite Island, Monte Bello Islands.
References—Lewis, 1914, p. 420.
Meggitt, 1924, p. 34.
Nybelin, 1917, p. 33.
Ortlepp, 1922, p. 610.

PROGAMOTAENIA ZSCHOKKEI (Janicki, 1905) Nybelin, 1917.
Host—Macropus sp.
Locality—New Guinea.
References—Janicki, 1905, p. 128; 1906A, p. 528.
Johnston, 1909, p. 518.
Lewis, 1914, p. 425.
Meggitt, 1924, p. 34.
Ortlepp, 1922, p. 610.

RAILLIETINA (PARONIELLA) MACROPA Ortlepp, 1922.
Host—Macropus brunii.
Location—Intestine.
Locality—Zool. Gdns., London.
References—Meggitt, 1924, p. 50.
Ortlepp, 1922, p. 602.

SPARGANUM REPTANS (Diesing, 1850).
Hosts—Didelphis brevicaudata.
Metachirus opossum.
Location—Occurs as larval form.
References—Diesing, 1850, p. 582.
Linstow, 1878, p. 64.
Meggitt, 1924, p. 158.
Parona, 1901, p. 2.

SPARGANUM sp. Johnston, 1909.

Host—Dasyurus viverrinus.
Location—Larval form in body cavity.
Locality—Sydney, New South Wales.
Reference—Johnston, 1909, p. 521.

SPARGANUM sp. (Parona, 1901).

Host—Chironectes minimus.
Location—Occurs as larval form.
Locality—São Paulo.
References—Meggitt, 1924, p. 158.
Parona, 1901, p. 2.

TAENIA GEOPHILOIDES Cobbold, 1879.

Host—Phascolarctos cinereus.
Locality—Brisbane, Australia.
References—Cobbold, 1879, p. 432.
Meggitt, 1924, p. 87.
Ortlepp, 1922, p. 611.

TAENIA KREFFTI Johnston, 1909.

Hosts—Macropus sp.
"Northern Wallaby."
Locality—Queensland, Australia.
References—Cobbold, 1879, p. 432.
Johnston, 1909, p. 518; 1912, p. 3.
Krefft, 1871, p. 218.
Meggitt, 1924, p. 89.
Ortlepp, 1922, p. 610.

TAENIA MASTERSII Krefft, 1871.

Host—Macropus sp.
Locality—Queensland, Australia.
References—Cobbold, 1879, p. 432.
Johnston, 1909, p. 519; 1912, p. 3.
Krefft, 1871, p. 221.
Meggitt, 1924, p. 90.
Ortlepp, 1922, p. 610.
Zschokke, 1898B, pp. 359, 376; 1899, p. 431.

TAENIA PHALANGISTAE Krefft, 1871.

Host—Trichosurus vulpecula.

Location—Intestine.

Locality—Queensland or New South Wales ; Australia.

References—Cobbold, 1879, p. 432.
Johnston, 1909, p. 520 ; 1912, p. 3.
Krefft, 1871, p. 221.
Linstow, 1889, p. 27.
Meggitt, 1924, p. 91.
Ortlepp, 1922, p. 611.
Zschokke, 1898B, pp. 359, 376 ; 1899, p. 431.

TRIPLOTAENIA MIRABILIS Boas, 1902.

Hosts—Macropus sp.
Petrogale penicillata.

Location—Intestine.

Locality—Australia.

References—Boas, 1902, p. 329.
Janicki, 1904A, p. 243 ; 1906A, p. 520.
Meggitt, 1924, p. 36.
Ortlepp, 1922, p. 610.
Spengel, 1905, p. 287.
Zschokke, 1904A, p. 293 ; 1904B, p. 60 ; 1907, p. 264.

NEMATODA.

ACANTHOCHEILONEMA AUSTRALE (Linstow, 1897) Baylis, 1925.

Hosts—Petrogale penicillata.
Trichosurus vulpecula.
Wallaby.

Location—" Stomach cavity " (probably abdominal cavity).

Locality—Torrens Creek, North Queensland ; Australia.

References—Baylis, 1925, p. 113.
Johnston, 1909, p. 519.
Leiper, 1911, p. 620.
Linstow, 1897, p. 610 ; 1905, p. 358.
Yorke & Maplestone, 1926, p. 426.

ACANTHOCHEILONEMA ROEMERI (Linstow, 1905) Baylis, 1925.
Hosts—Macropus antilopinus.
Macropus giganteus.
Location—Leg joints.
Locality—St. George District, Queensland ; Australia.
References—Baylis, 1925, p. 114.
Johnston, 1909, p. 517.
Linstow, 1905, p. 356 ; 1906A, p. 7.
Yorke & Maplestone, 1926, p. 427.

ASCARIS sp. Krefft, 1871.
Host—Perameles nasuta.
Locality—Australia.
References—Johnston, 1909, p. 521.
Krefft, 1871, p. 212.

ASPIDODERA HARWOODI Chandler, 1932.
Host—Didelphis marsupialis virginiana.
Location—Caecum.
Locality—Houston, Texas, U.S.A.
Reference—Chandler, 1932, p. 8.

ASPIDODERA RAILLIETI Travassos, 1913.
Host—Didelphis marsupialis aurita.
Location—Caecum.
Locality—Brazil.
References—Travassos, 1913, p. 306.
Yorke & Maplestone, 1926, p. 220.

ASPIDODERA SCOLECIFORMIS (Diesing, 1851).
Hosts—Didelphis domestica.
Marmosa murina.
Location—Intestine, caecum.
Locality—Brazil.
References—Cobbold, 1879, p. 321.
Diesing, 1851, p. 208.
Linstow, 1878, p. 64.
Stiles & Hassall, 1905, p. 88.
Travassos, 1913, p. 305.
Yorke & Maplestone, 1926, p. 220.

ASPIDODERA SUBULATA (Molin, 1860).

Host—Metachirus nudicaudatus.
Location—Stomach, intestine.
Locality—Ypanema.
References—Cobbold, 1879, p. 434.
Linstow, 1889, p. 27.
Molin, 1860, p. 514.
Railliet & Henry, 1912, p. 257.
Travassos, 1913, p. 305.
Yorke & Maplestone, 1926, p. 220.

AUSTROSTRONGYLUS MACROPODIS Chandler, 1924.

Host—Macropus ruficollis bennetti.
Location—Upper part of small intestine.
Locality—Australia.
References—Chandler, 1924, p. 160.
Yorke & Maplestone, 1926, p. 138.

BREINLIA DENDROLAGI Solomon, 1933.

Host—Dendrolagus inustus.
Location—Peritoneal cavity.
Locality—Zool. Gdns., London.
Reference—Solomon, 1933, p. 101.

BREINLIA TRICHOSURI (Breinl, 1911).

Host—Trichosurus vulpecula.
Locality—North Queensland.
References—Breinl, 1913, p. 39.
Yorke & Maplestone, 1926, p. 401.

CAPILLARIA AURITAE Travassos, 1914.

Host—Didelphis marsupialis aurita.
Location—Small intestine.
Locality—Brazil.
References—Travassos, 1914B, p. 429 ; 1915A, p. 161.
Yorke & Maplestone, 1926, p. 25.

CLOACINA DAHLI Linstow, 1898.

Host—Macropus browni.
Location—Alimentary canal.

Locality—Ralum ; Bismark Archipelago.

References—Johnston, 1909, p. 518.
Linstow, 1898A, p. 286.
Stiles & Hassall, 1905, p. 94.
Yorke & Maplestone, 1926, p. 176.

CRUZIA TENTACULATA (Rudolphi, 1819) Travassos, 1917.

Hosts—Didelphis domestica.
Didelphis marsupialis aurita.
Didelphis marsupialis karkinophaga.
Didelphis marsupialis virginiana.
Didelphis quoaiquiqua.
Marmosa murina.
Metachirus nudicaudatus.
Metachirus opossum.
Philander lanigera.
Philander philander.

Location—Intestine, caecum.

Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.; Houston, Texas, U.S.A.; Pennsylvania; Brazil.

References—Chandler, 1932, p. 10.
Cobbold, 1879, p. 433.
Diesing, 1851, p. 147 ; 1861, p. 655.
Dikmans, 1931, p. 3.
Dujardin, 1845, p. 168.
Leidy, 1856, p. 51.
Linstow, 1878, p. 64.
Rudolphi, 1819, p. 658.
Schneider, 1866, p. 115.
Stiles & Hassal, 1894, p. 340.
Travassos, 1917, p. 100 ; 1922, p. 88.
Yorke & Maplestone, 1926, p. 242.

DIROFILARIA WEBSTERI (Cobbold, 1879).

Hosts—Macropus giganteus.
Kangaroo.

Location—Knee joint.
Locality—Queensland ? ; New South Wales ; Australia.
References—Cobbold, 1879, p. 433.
Fletcher, 1883, p. 388.
Johnston, 1909, p. 516 ; 1911A, p. 47.
Linstow, 1889, p. 27 ; 1905, p. 357.
Railliet & Henry, 1910, p. 251.
Yorke & Maplestone, 1926, p. 395.

ECHINONEMA CINCTA Linstow, 1898.
Host—Isoodon obesula.
Location—Intestine.
Locality—Queensland ; Australia.
References—Gemmill & Linstow, 1902, p. 113.
Johnston, 1909, p. 521 ; 1911A, p. 50.
Linstow, 1898B, p. 469.
Parona, 1900, p. 194.
Yorke & Maplestone, 1926, p. 348.

ECHINONEMA sp. Parona, 1900.
Host—Didelphis marsupialis azarae.
Location—Intestine.
Locality—Museum, Buenos Aires.
Reference—Parona, 1900, p. 194.

ECHINORHYNCHUS MICROCEPHALUS Rudolphi, 1819.
Hosts—Didelphis marsupialis aurita.
Didelphis marsupialis karkinophaga.
Didelphis marsupialis virginiana.
Didelphis sp.
Marmosa murina.
Philander philander.
Location—Mesentery.
Locality—Brazil ; U.S.A.
References—Cobbold, 1879, p. 434.
Diesing, 1851, p. 20.
Dujardin, 1845, p. 504.
Ihering, 1902, p. 45.
Leidy, 1856, p. 48.

Linstow, 1878, p. 65.
Luehe, 1905, p. 254.
Porta, 1909, p. 256.
Rudolphi, 1819, p. 665.
Travassos, 1915B, p. 105 ; 1915C, p. 137.
Westrumb, 1821, p. 3.

FILARIA DENTIFERA Linstow, 1898.
Host—Trichosurus vulpecula.
Location—Body cavity.
Locality—Queensland or New South Wales ; Australia.
References—Johnston, 1909, p. 520.
Linstow, 1898B ; 1905, p. 358.

FILARIA sp. Eisig, 1869.
Host—Macropus ruficollis bennetti.
Location—Pericardium.
Locality—Heidelberg Zool. Inst. ; Tasmania.
References—Eisig, 1869, p. 99.
Johnston, 1909, p. 518.
Linstow, 1878, p. 64 ; 1905, p. 357.

FILARIA sp. Johnston, 1910.
Host—Dendrolagus bennettianus.
Location—Subcutaneous tissues.
Locality—North Queensland.
References—Johnston, 1910, p. xii ; 1911A, p. 49.

FILARIA sp. Johnston, 1910.
Host—Onychogalea frenata.
Location—In nodules in subcutaneous tissues.
Locality—Gippsland, Victoria, Australia.
References—Johnston, 1910, p. xii ; 1911A, p. 49

FILARIA sp. Johnston, 1910.
Host—Trichosurus caninus.
Location—Peritoneum.
Locality—Gosford District, New South Wales.
References—Johnston, 1910, p. xvii ; 1911A, p. 49.

FILARIA sp. Plimmer, 1912.

Host—Metachirus nudicaudatus.

Location—Blood.

Locality—Zool. Gardens, London.

References—Plimmer, 1912A, p. 407 ; 1912B, p. 137.

FILARIA sp. Plimmer, 1912.

Host—Onychogalea frenata.

Location—Blood.

Locality—Zool. Gardens, London.

References—Plimmer, 1912A, p. 407 , 1912B, p. 137.

GLOBOCEPHALOIDES MACROPODIS Yorke & Maplestone, 1926.

Host—Macropus sp.

Reference—Yorke & Maplestone, 1926, p. 174.

GNATHOSTOMA DIDELPHYS Chandler, 1932.

Host—Didelphis marsupialis virginiana.

Location—Liver.

Locality—Houston, Texas, U.S.A.

Reference—Chandler, 1932, p. 10.

GNATHOSTOMA TURGIDUM Stossich, 1902.

Hosts—Didelphis marsupialis aurita.
Didelphis marsupialis azarae.
Didelphis marsupialis virginiana.

Location—Stomach.

Locality—Republic of Argentina ; Brazil ; Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.

References—Baylis & Lane, 1920, p. 301.
Braun, 1915, p. 304.
Dikmans, 1931, p. 2.
Stossich, 1902, p. 13.
Travassos, 1925, p. 5.
Yorke & Maplestone, 1926, p. 340.

HELIGMOSOMUM DIDELPHE (Travassos, 1914) Travassos, 1918.

Host—Didelphis marsupialis aurita.

Location—Small intestine.

Locality—Brazil.

References—Travassos, 1914A, p. 326; 1919, p. 197; 1921, pp. 35, 89.
Yorke & Maplestone, 1926, p. 142.

HETERAKIS PARADOXA Linstow, 1906.

Host—Marmosa murina.

Location—Intestine.

References—Linstow, 1906B, p. 750.
Railliet & Henry, 1914, p. 676.
Yorke & Maplestone, 1926, p. 216.

LABIOSTRONGYLUS LABIOSTRONGYLUS Yorke & Maplestone, 1926.

Host—Macropus sp.

Reference—Yorke & Maplestone, 1926, p. 67.

LABIOSTRONGYLUS LONGISPICULARIS Wood, 1929.

Host—Macropus robustus woodwardi.

Location—Stomach.

Locality—? Western Australia.

Reference—Wood, 1929A, p. 550.

LAGOCHILASCARIS TURGIDA (Stossich, 1902).

Host—Metachirus crassicaudatus.

Locality—Buenos Aires.

References—Stossich, 1902, p. 2.
Yorke & Maplestone, 1926, p, 261.

MACROPOSTRONGYLUS AUSTRALIS Yorke & Maplestone, 1926.

Host—Macropus sp.

Locality—Queensland.

Reference—Yorke & Maplestone, 1926, p. 75.

MACROPOSTRONGYLUS BAYLISI Wood, 1930.

Host—Macropus robustus woodwardi.

Location—Caecum.

Locality—Western Australia.

Reference—Wood, 1930, p. 209.

MACROPOSTRONGYLUS MACROPOSTRONGYLUS Yorke & Maplestone, 1926.

Host—Macropus sp.

Locality—Queensland.

Reference—Yorke & Maplestone, 1926, p. 75.

MACROPOSTRONGYLUS YORKEI Baylis, 1927.
Host—Macropus sp.
Locality—Townsville, North Queensland.
Reference—Baylis, 1927, p. 215.

NICOLLINA SARCOPHILI Cameron, 1931.
Host—Sarcophilus harrisii.
Location—Intestine.
Locality—Scottish Zool. Park, Edinburgh.
References—Cameron, 1931, p. 153.
Baylis, 1930A, p. 10; 1930B, p. 550.

OESOPHAGOSTOMUM sp. Dikmans, 1931.
Host—Didelphis marsupialis virginiana.
Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.
Reference—Dikmans, 1931, p. 3.

OSTERTAGIA CALLIS (Travassos, 1914) Travassos, 1918.
Host—Didelphis marsupialis aurita.
Location—Small intestine.
Locality—Brazil.
References—Travassos, 1914A, p. 325; 1919, p. 193; 1921, pp. 24, 61
Yorke & Maplestone, 1926, p. 125.

PHARYNGOSTRONGYLUS AUSTRALIS (Mönnig, 1926) Wood, 1929.
Hosts—Macropus robustus woodwardi.
Macropus rufus.
Location—Stomach.
Locality—Western Australia.
References—Mönnig, 1926, p. 293; 1927, p. 265.
Wood, 1929B, p. 552.

PHARYNGOSTRONGYLUS MACROPODIS Yorke & Maplestone, 1926.
Host—Macropus sp.
Reference—Yorke & Maplestone, 1926, p. 67.

PHARYNGOSTRONGYLUS WOODWARDI Wood, 1930.
Host—Macropus robustus woodwardi.
Location—Stomach.

Locality—Western Australia.
Reference—Wood, 1930, p. 213.

PHYSALOPTERA TURGIDA Rudolphi, 1819.
Hosts—Didelphis marsupialis azarae.
Didelphis marsupialis karkinophaga.
Didelphis marsupialis virginiana.
Marmosa murina.
Metachirus crassicaudatus.
Metachirus nudicaudatus.
Locality—Buenos Aires, Brazil ; Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La. ; Houston, Texas.
References—Chandler, 1932, p. 10.
Cobbold, 1879, p. 433.
Diesing, 1851, p. 233.
Dikmans, 1931, p. 3.
Leidy, 1856, p. 53 ; 1886, p. 312 ; 1904, p. 98.
Linstow, 1878, p. 64.
Parona, 1900, p. 195.
Rudolphi, 1819, p. 644.
Schneider, 1866, p. 62.
Stossich, 1889, p. 43 ; 1902, p. 10.
Yorke & Maplestone, 1926, p. 355.

PROTOSPIRURA MARSUPIALIS Baylis, 1927.
Host—Opossum—probably Trichosurus vulpecula.
Locality—Townsville, North Queensland.
Reference—Baylis, 1927, p. 220.

SETARIA SPELAEA (Leidy, 1875).
Hosts—Australian Wallaby.
Macropus sp.
Location—Abdominal cavity.
Locality—Zool. Gdns., Philadelphia, U.S.A. ; Australia
References—Cobbold, 1879, p. 433.
Johnston, 1909, p. 518.
Leidy, 1875B, p. 17 ; 1904, p. 137.
Railliet & Henry, 1911, p. 388.
Yorke & Maplestone, 1926, p. 423.

SPIROCERCA HEYDONI Baylis, 1927.

Host—Dasyurus sp.

Location—Cysts in wall of stomach and intestine; also a few free in stomach.

Locality—Cairns, North Queensland.

Reference—Baylis, 1927, p. 221.

SPIROSTRONGYLUS SPIROSTRONGYLUS Yorke & Maplestone, 1926.

Host—Macropus sp.

Reference—Yorke & Maplestone, 1926, p. 70.

TRICHOSTRONGYLUS ASYMMETRICUS Cameron, 1926.

Hosts—Macropus robustus woodwardi.
Macropus ruficollis bennetti.

Location—Stomach and intestine.

Locality—Western Australia.

References—Cameron, 1926, p. 23.
Wood, 1930, p. 216.

TRICHOSTRONGYLUS AUSTRALIS Wood, 1930.

Host—Macropus robustus woodwardi.

Location—Stomach and intestine.

Locality—Western Australia.

Reference—Wood, 1930, p. 218.

TRICHOSTRONGYLUS DISSIMILIS Wood, 1930.

Host—Macropus robustus woodwardi.

Location—Stomach and intestine.

Locality—Western Australia.

Reference—Wood, 1930, p. 217.

TRICHOSTRONGYLUS sp. Dikmans, 1931.

Host—Didelphis marsupialis virginiana.

Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.

Reference—Dikmans, 1931, p. 3.

TRICHURIS MINUTA (Rudolphi, 1819).

Hosts—Didelphis domestica.
Didelphis marsupialis azarae.

Didelphis marsupialis karkinophaga.
Didelphis marsupialis virginiana.
Marmosa murina.
Metachirus nudicaudatus.

Location—Intestine and caecum.
Locality—Brazil.
References—Chandler, 1930, p. 199.
Cobbold, 1879, p. 433.
Dujardin, 1845, p. 40.
Heine, 1900, p. 780.
Rudolphi, 1819, p. 638.

TRICHURIS PERAMELIS Baylis, 1932.

Host—Isoodon obesula.
Location—Intestine.
Locality—Rollingstone, North Queensland.
Reference—Baylis, 1932, p. 31.

TRICHURIS sp. Dikmans, 1931.

Host—Didelphis marsupialis virginiana.
Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.
Reference—Dikmans, 1931, p. 3.

VIANNAIA BURSOBSCURA Dikmans, 1931.

Host—Didelphis marsupialis virginiana.
Location—Small intestine.
Locality—Lab., Zool. Div., Bur. Anim. Ind., U.S. Dept. Agric., Jeanerette, La.
Reference—Dikmans, 1931, p. 1.

VIANNAIA CONSPICUA Travassos, 1914.

Host—Metachirus opossum.
Location—Small intestine.
Locality—Brazil.
References—Travassos, 1914A, p. 326; 1919, p. 204; 1921, pp. 37, 98.
Yorke & Maplestone, 1926, p. 149.

VIANNAIA HAMATA Travassos, 1914.

Host—Didelphis marsupialis aurita.

Location—Small intestine.

Locality—Brazil.

References—Travassos, 1914A, p. 327; 1919, p. 204; 1921, pp. 37, 99.
Yorke & Maplestone, 1926, p. 150.

VIANNAIA PUSILLA Travassos, 1914.

Host—Didelphis marsupialis aurita.

Location—Small intestine.

Locality—Brazil.

References—Travassos, 1914A, p. 327; 1919, p. 204; 1921, pp. 37, 99.
Yorke & Maplestone, 1926, p. 150.

VIANNAIA VIANNAI Travassos, 1914.

Host—Didelphis marsupialis aurita.

Location—Small intestine.

Locality—Brazil.

References—Travassos, 1914A, p. 326; 1919, p. 203; 1921, pp. 36, 97.
Yorke & Maplestone, 1926, p. 149.

ZONIOLAIMUS BREVICAUDATUS Cobb, 1898.

Host—Macropus sp.

References—Cobb, 1898, p. 440.
Yorke & Maplestone, 1926, p. 176.

ZONIOLAIMUS SETIFERA Cobb, 1898.

Hosts—Macropus sp.
Macropus ualabatus.

Location—Stomach.

Locality—Moss Vale, New South Wales, Australia.

References—Cobb, 1898, p. 312.
Johnston, 1909, p. 518.
Yorke & Maplestone, 1926, p. 176.

ACANTHOCEPHALA.

GIGANTORHYNCHUS SEMONI Linstow, 1898.

Hosts—Isoodon obesula.

Perameles nasuta.

Location—Intestine.

Locality—Queensland ; Sydney, New South Wales ; Australia.

References—Johnston, 1909, p. 521 ; 1911A, p. 50.
Linstow, 1898B, p. 471.
Porta, 1908, p. 276.
Zschokke, 1904A, p. 293 ; 1904B, p. 61.

GIGANTORHYNCHUS sp. Johnston, 1910.

Host—Phascogale penicillata.

Location—Intestine.

Locality—New South Wales.

Reference—Johnston, 1911A, p. 50.

NOTES.

The trematodes for which marsupials are the definitive hosts call for no special comment as the majority appear to be specific to their hosts except *Fasciola hepatica* which has been reported from various wild and domesticated mammals.

Amongst the cestodes four forms require to be commented upon. The form *Taenia geophiloides* Cobbold, 1879 seems to be doubtfully considered by Meggitt (1924, p. 28), at least, as a synonym of *Bothriocephalus marginatus* Krefft, 1871 while Johnston (1912, p. 3) considers it probably belongs to the Anoplocephalidae rather than the Taeniidae.

Ortlepp (1922) mentions that the form *Moniezia bipapillosa* (Leidy, 1875), a recognized synonym of which is *Taenia bipapillosa*, should be considered as synonymous with *Hepatotaenia diaphana* (Zschokke, 1907) Nybelin, 1917. Both forms, however, have here been listed.

With regard to *Triplotaenia mirabilis* Boas, 1902, Janicki (1904A, p. 243) considers this species a monstrosity.

Amongst the nematodes the filarial forms require some comment. *Dirofilaria websteri* (Cobbold, 1879) is considered as probably synonymous with *Acanthocheilonema roemeri* (Linstow, 1905) by Baylis (1925). In this list of parasites seven species of the genus *Filaria* are mentioned only one of which is cited specifically. The remaining six, only referred to in the literature as " *Filaria* sp.", have in each case the author and date

added. The only criterion for differentiation is the host record although it should be pointed out that such a procedure is not strictly sound as it is possible for more than one host to harbour the same form of *Filaria*.

The name *Echinorhynchus tortuosus* Leidy, 1850 which may be met with in literature references is recognized as a synonym of *Echinorhynchus microcephalus* Rudolphi, 1819.

It may be useful here to summarize the numbers of helminths parasitizing marsupials. There are 19 species of trematodes distributed among 11 genera, 45 species of cestodes among 20 genera, 61 species of nematodes among 32 genera and 2 species of acanthocephalids in 1 genus ; or, in the aggregate 127 species of helminths among 64 genera.

Marsupials as Reservoir Hosts.

During the accumulation of information on the parasites of marsupials the possibility of these mammals acting as reservoir hosts was not lost sight of but the results have demonstrated that, in the majority of cases, the helminths reported from marsupials appear to be specific to them. In only three cases can it be said with any degree of certainty that other wild as well as domesticated animals harbour the same parasites as marsupials.

The Liver Fluke, *Fasciola hepatica*, is recorded as occurring in Kangaroos, Wallabies, *Macropus giganteus* and *M. ruficollis*. This trematode is also known to infest various mammals including economically important forms such as cattle, sheep, horses, pigs, as well as goats, asses, deer and rabbits. It is also not infrequently found in man in certain parts of the world. That the native marsupial fauna is capable of assisting in the spread of fluke disease seems possible and therefore in this capacity marsupials should be considered as reservoir hosts. Similarly in the case of Hydatid, *Echinococcus granulosus*, this parasite besides occurring in at least six species of *Macropus* is also met with, as the adult form, in canines, and as the larval form in cattle, sheep, pigs, horses, deer, goats, mules and rabbits as well as in human beings. The third cestode common to marsupials and other mammals is *Sparganum reptans*. Its importance is rather insignificant, however, as none of the hosts is of the domesticated variety.

To those apprehensive lest marsupials should function as disseminators of parasitic infections it is therefore reassuring to know that, of the parasites reported from them, less than 3 per cent. occur, so far as is known, in other economically important animals.

Host-List of Helminth Parasites.

The following Host-list of the helminth parasites of marsupials is an alphabetical arrangement of the recognized scientific names of the species of marsupials to which reference has been made in the relevant literature together with their principal synonyms and popular names. The synonyms and popular names listed refer only to the accepted names of forms treated in this memorandum. Under each recognized species of host will be found the following information : The author and date of the host specific name are on the first line. Below this are given respectively the synonyms (if any), the geographical distribution or habitat of the host, and the parasites pertaining to that species of marsupial. The parasites are listed in alphabetical order, each species of helminth having inserted before it a letter indicative of the Class of vermes to which it belongs. Thus T indicates the Class Trematoda, C, Cestoda, N, Nematoda and A, Acanthocephala. This list is intended for use in conjunction with the foregoing Parasite-list in which the arrangement is under parasites and where fuller information is obtainable about the actual helminths together with literature references.

J. N. Oldham.

Host-List of Parasites Arranged under Scientific Names of Marsupials.

Agile Wallaby, *see* Macropus agilis.
American Opossum, *see* Didelphis marsupialis.
Antilopine Kangaroo, *see* Macropus antilopinus.
Aru Island Wallaby, *see* Macropus brunii.
Australian Naked-nosed Wombat, *see* Phascolomis mitchelli.
Azara's Opossum, *see* Didelphis marsupialis azarae.
Bennett's Tree-kangaroo, *see* Dendrolagus bennettianus.

Bennett's Wallaby, *see* Macropus ruficollis bennetti.
Black Cuscus, *see* Phalanger ursinus.
Black-striped Wallaby, *see* Macropus dorsalis.
Black-tailed Wallaby, *see* Macropus ualabatus.
Black Wallaby, *see* Macropus ualabatus.
Bridled Nail-tailed Wallaby, *see* Onychogalea frenata.
Brush-tailed Pouched-Mouse, *see* Phascogale penicillata.
Brush-tailed Rock-Wallaby, *see* Petrogale penicillata.
Chilian Opossum, *see* Marmosa elegans.

CHIRONECTES MINIMUS (Zimmermann, 1780).
Synonymy—*Chironectes palmata.*
Didelphys palmata.
Distribution—Guatemala to South Brazil.
Parasites—
T. Rhopalias baculifer.
T. Rhopalias coronatus.
C. Sparganum sp. (Parona, 1901).

Chironectes palmata, see *Chironectes minimus*.

Common Three-striped Opossum, *see* Didelphis americana.

Crab-eating Opossum, *see* Didelphis marsupialis karkinophaga.

Dama Wallaby, *see* Macropus eugenii.

DASYURUS sp.
Distribution—North Queensland.
Parasites—
N. Spirocerca heydoni.

Dasyurus ursinus, see *Sarcophilus harrisii*.

DASYURUS VIVERRINUS (Shaw, 1800).
Distribution—Australia: New South Wales, South Australia and Tasmania.
Parasites—
T. Harmostomum dasyuri.
T. Mehlisia acuminata.
C. Sparganum sp. Johnston, 1909.

De Bruyn's Wallaby, *see* Macropus brunii.

DENDROLAGUS BENNETTIANUS De Vis, 1886.
Distribution—Australia : Queensland.
Parasites—
N. Filaria sp. Johnston, 1910.

DENDROLAGUS INUSTUS Schlegel & S. Müller 1839.
Distribution—New Guinea.
Parasite—
N. Breinlia dendrolagi.

Derbian Wallaby, *see* Macropus eugenii.

DIDELPHIS AMERICANA (Müller, 1776).
Synonymy—*Didelphis bistriata* (?)
Didelphys tristriatus.
Peramys americana.
Peramys tristriata.
Distribution—Brazil.
Parasites—
C. Linstowia brasiliensis.
C. Linstowia jheringi.

Didelphis bistriata, see (?) *Didelphis americana.*

DIDELPHIS BREVICAUDATA Erxleben, 1777.
Synonymy—*Didelphys brachyura.*
Distribution—Guiana and Brazil.
Parasites—
C. Sparganum reptans.

DIDELPHIS DOMESTICA Wagner, 1842.
Distribution—Brazil.
Parasites—
N. Aspidodera scoleciformis.
N. Cruzia tentaculata.
N. Trichuris minuta.

DIDELPHIS GOAGNIA (see explanatory notes).
Parasites—
C. Bothriocephalus sp. Janicki, 1904.

DIDELPHIS MARSUPIALIS Linnaeus, 1758.

Distribution—America : from Northern United States southwards to Chile, Paraguay and Southern Brazil.

Parasites—

C. Bothriocephalus didelphidis.

DIDELPHIS MARSUPIALIS AURITA Wied, 1826.

Synonymy—*Didelphys aurita.*

Distribution—South America : South-eastern Brazil.

Parasites—

N. Aspidodera raillieti.
N. Capillaria auritae.
N. Cruzia tentaculata.
N. Echinorhynchus microcephalus.
N. Gnathostoma turgidum.
T. Harmostomum opisthotrias.
N. Heligmosomum didelphe.
T. Levinseniella jägerskioldi.
N. Ostertagia callis.
T. Rhopalias coronatus.
T. Rhopalias horridus.
N. Viannaia hamata.
N. Viannaia pusilla.
N. Viannaia viannai.

DIDELPHIS MARSUPIALIS AZARAE Temminck, 1827.

Synonymy—*Didelphys azarae.*

Distribution—South America : from Colombia and Chile to Brazil.

Parasites—

C. Bothriocephalus didelphidis.
N. Echinonema sp. Parona, 1900.
N. Gnathostoma turgidum.
N. Physaloptera turgida.
T. Plagiorchis didelphidis.
N. Trichuris minuta.

DIDELPHIS MARSUPIALIS KARKINOPHAGA Zimmermann, 1780.

Synonymy—*Didelphys cancrivora.*

Distribution—Tropical America.

Parasites—

N. Cruzia tentaculata.
N. Echinorhynchus microcephalus.
T. Hemistomum pedatum.
N. Physaloptera turgida.
T. Rhopalias coronatus.
N. Trichuris minuta.

DIDELPHIS MARSUPIALIS VIRGINIANA Kerr, 1792.

Synonymy—*Didelphys virginiana.*

Distribution—North America, from Long Island and the Great Lakes to Oklahoma and Texas. Introduced into California.

Parasites—

N. Aspidodera harwoodi.
N. Cruzia tentaculata.
N. Echinorhynchus microcephalus.
T. Echinostomum sp. Dikmans, 1931.
N. Gnathostoma didelphys.
N. Gnathostoma turgidum.
T. Harmostomum opisthotrias.
T. Harmostomum opisthotrias var. virginiana.
T. Harmostomum sp. Dikmans, 1931.
C. Mesocestoides sp. Dikmans, 1931.
T. Neodiplostomum lucidum.
N. Oesophagostomum sp. Dikmans, 1931.
N. Physaloptera turgida.
T. Proalaria variabilis.
T. Rhopalias coronatus.
T. Rhopalias macracanthus.
T. Rhopalias sp. Dikmans, 1931.
N. Trichostrongylus sp. Dikmans, 1931.
N. Trichuris minuta.
N. Trichuris sp. Dikmans, 1931.
N. Viannaia bursobscura.

DIDELPHIS QUOAIQUIQUA (see explanatory notes).

Parasites—

N. Cruzia tentaculata.

DIDELPHIS sp.

Parasites—

C. Bothriocephalus sp. Janicki, 1904.

N. Echinorhynchus microcephalus.

Didelphys aurita, see *Didelphis marsupialis aurita*.

Didelphys azarae, see *Didelphis marsupialis azarae*.

Didelphys brachyura, see *Didelphis brevicaudata*.

Didelphys cancrivora, see *Didelphis marsupialis karkinophaga*.

Didelphys cayopollin, see *Marmosa murina*.

Didelphys crassicaudata, see *Metachirus crassicaudatus*.

Didelphys derbiana, see *Philander lanigera*.

Didelphys dorsigera, see *Marmosa murina*.

Didelphys elegans, see *Marmosa elegans*.

Didelphys lanigera, see *Philander lanigera*.

Didelphys murina, see *Marmosa murina*.

Didelphys myosurus, see *Metachirus nudicaudatus*.

Didelphys nudicaudata, see *Metachirus nudicaudatus*.

Didelphys opossum, see *Metachirus opossum*.

Didelphys palmata, see *Chironectes minimus*.

Didelphys philander, see *Philander philander*.

Didelphys quica, see *Metachirus opossum*.

Didelphys tristriatus, see *Didelphis americana*.

Didelphys virginiana, see *Didelphis marsupialis virginiana*.

Eugene Island Wallaby, *see* Macropus eugenii.

Fawn Nail-tailed Wallaby, *see* Onychogalea unguifera.

Flinders Island Wombat, *see* Phascolomis ursinus.

Forester, *see* Macropus giganteus.

Four-spotted Opossum, *see* Metachirus opossum.

Gamba Opossum, *see* Didelphis marsupialis aurita.

Great Grey Kangaroo, *see* Macropus giganteus.

Grizzled Grey Tree-Kangaroo, *see* Dendrolagus inustus.

Halmaturus, see *Macropus*.

Halmaturus bennettii, see *Macropus ruficollis bennetti*.

Herbert-River Phalanger, *see* Pseudochirus herbertensis.

ISOODON OBESULA (Shaw, 1793).
Synonymy—*Perameles obesula*.
Distribution—Australia : widely distributed.
Parasites—
N. Echinonema cincta.
A. Gigantorhynchus semoni.
T. Harmostomum simile.
C. Linstowia echidnae.
C. Linstowia semoni.
N. Trichuris perameles.

Kangaroo (Unspecified).
Parasites—
N. Dirofilaria websteri.
T. Fasciola hepatica.

Koala, *see* Phascolarctos cinereus.

LAGORCHESTES CONSPICILLATUS Gould, 1841.
Distribution—Islands off the North-west coast of Australia.
Parasites—
C. Cittotaenia villosa.
C. Progamotaenia lagorchestis.

Long-eared Opossum, *see* Trichosurus vulpecula.

Long-nosed Bandicoot, *see* Perameles nasuta.

MACROPUS AGILIS (Gould, 1841).
Distribution—Northern Australia : some islands off coast and Papua.
Parasites—
C. Hepatotaenia fellicola.

MACROPUS ANTILOPINUS (Gould, 1841).

Distribution—Australia : Arnhem Land, Northern Territory.

Parasites—

N. Acanthocheilonema roemeri.

Macropus bennettii, see *Macropus ruficollis bennetti*.

MACROPUS BROWNI (Ramsay, 1877).

Distribution—New Britain group of islands ; Eastern and South-eastern New Guinea.

Parasites—

N. Cloacina dahli.

MACROPUS BRUNII (Schreber, 1778).

Distribution—Aru and Kei Islands.

Parasites—

C. Raillietina macropa.

Macropus derbianus, see *Macropus eugenii*.

MACROPUS DORSALIS (Gray, 1837).

Distribution—Eastern Australia.

Parasites—

C. Echinococcus granulosus.

MACROPUS EUGENII (Desmarest, 1817).

Synonymy—*Macropus derbianus*.

Distribution—South-western Australia and islands off coast.

Parasites—

C. Echinococcus granulosus.

C. Hepatotaenia festiva.

MACROPUS GIGANTEUS (Zimmermann, 1777).

Synonymy—*Macropus major*.

Distribution—Australia.

Parasites—

N. Acanthocheilonema roemeri.

N. Dirofilaria websteri.

C. Echinococcus granulosus.

T. Fasciola hepatica.

C. Hepatotaenia festiva.

Macropus major, see *Macropus giganteus*.

Macropus robustus Gould, 1840.

Distribution—Eastern Australia: mountain country from South Queensland to New South Wales.

Parasites—

C. Echinococcus granulosus.

C. Hepatotaenia festiva.

Macropus robustus woodwardi Thomas, 1901.

Synonymy—*Macropus woodwardi*.

Distribution—Western Australia: Murchison District.

Parasites—

N. Labiostrongylus longispicularis.

N. Macropostrongylus baylisi.

N. Pharyngostrongylus australis.

N. Pharyngostrongylus woodwardi.

N. Trichostrongylus asymmetricus.

N. Trichostrongylus australis.

N. Trichostrongylus dissimilis.

Macropus ruficollis (Desmarest, 1817).

Distribution—South-eastern Australia.

Parasites—

T. Fasciola hepatica.

Macropus ruficollis bennetti Waterhouse, 1837.

Synonymy—*Halmaturus bennettii*.

Macropus bennettii.

Distribution—Tasmania.

Parasites

N. Austrostrongylus macropodis.

N. Filaria sp. Eisig, 1869.

N. Trichostrongylus asymmetricus.

Macropus rufus (Desmarest, 1822).

Distribution—Australia: inland, open plain country.

Parasites—

N. Pharyngostrongylus australis.

MACROPUS sp.

Parasites—

C. Bothriocephalus marginatus.

N. Globocephaloides macropodis.

N. Labiostrongylus labiostrongylus.

N. Macropostrongylus australis.

N. Macropostrongylus macropostrongylus.

N. Macropostrongylus yorkei.

N. Pharyngostrongylus macropodis.

C. Progamotaenia zschokkei.

N. Setaria spelaea.

N. Spirostrongylus spirostrongylus.

C. Taenia kreffti.

C. Taenia mastersii.

C. Triplotaenia mirabilis.

N. Zoniolaimus brevicaudatus.

N. Zoniolaimus setifera.

MACROPUS THETIDIS (Lesson, 1827).

Distribution—South-eastern Australia.

Parasites—

C. Echinococcus granulosus.

MACROPUS UALABATUS (Lesson & Garnier, 1826).

Distribution—Eastern Australia.

Parasites—

C. Bancroftiella tenuis.

C. Echinococcus granulosus.

N. Zoniolaimus setifera.

Macropus woodwardi, see *Macropus robustus woodwardi*.

MARMOSA ELEGANS (Waterhouse, 1839).

Synonymy—*Didelphys elegans*.

Distribution—South America : Chile.

Parasites—

C. Oochoristica bivittata.

C. Oochoristica didelphidis.

C. Oochoristica marmosae.

C. Oochoristica murina.

MARMOSA MURINA (Linnaeus, 1758).

Synonymy—*Didelphys cayopollin.*
Didelphys dorsigera.
Didelphys murina.

Distribution—South and Central America: from Brazil to as far north as Mexico.

Parasites—

N. Aspidodera scoleciformis.
N. Cruzia tentaculata.
N. Echinorhynchus microcephalus.
N. Heterakis paradoxa.
C. Oochoristica bivittata.
C. Oochoristica didelphidis.
C. Oochoristica murina.
N. Physaloptera turgida.
N. Trichuris minuta.

Marsupial Wolf, *see* Thylacinus cynocephalus.

Merian's Opossum, *see* Marmosa murina.

METACHIRUS CRASSICAUDATUS (Desmarest, 1804).

Synonymy—*Didelphys crassicaudata.*

Distribution—South America: Argentina, Brazil and the Guianas.

Parasites—

N. Lagochilascaris turgida.
N. Physaloptera turgida.

METACHIRUS NUDICAUDATUS (Geoffroy, 1803).

Synonymy—*Didelphys myosurus.*
Didelphys nudicaudata.

Distribution—South and Central America (Brazil to Costa Rica).

Parasites—

N. Aspidodera subulata.
N. Cruzia tentaculata.
N. Filaria sp. Plimmer, 1912.
T. Hemistomum pedatum.
N. Physaloptera turgida.
T. Rhopalias coronatus.
T. Rhopalias horridus.
N. Trichuris minuta.

METACHIRUS OPOSSUM (Linnaeus, 1758).

Synonymy—*Didelphys opossum.*

Didelphys quica.

Distribution—South and Central America to as far north as Mexico.

Parasites—

N. Cruzia tentaculata.

T. Rhopalias coronatus.

T. Rhopalias horridus.

C. Sparganum reptans.

N. Viannaia conspicua.

Mitchell's Wombat, *see* Phascolomis mitchelli.

Murine Opossum, *see* Marmosa murina.

Native Cat, *see* Dasyurus sp.

Native Bear, *see* Phascolarctos cinereus.

North-Australian Bandicoot, *see* Perameles macrura.

Northern Wallaby, see explanatory notes.

Parasites—

C. Taenia kreffti.

ONYCHOGALEA FRENATA (Gould, 1840).

Distribution—Eastern Australia.

Parasites—

N. Filaria sp. Johnston, 1910.

N. Filaria sp. Plimmer, 1912.

C. Progamotaenia bancrofti.

ONYCHOGALEA UNGUIFERA (Gould, 1840).

Distribution—North-western and Northern Australia.

Parasites—

C. Hepatotaenia festiva.

C. Progamotaenia bancrofti.

Opossum, includes Didelphis, Marmosa, Metachirus, Philander, Pseudochirus and Trichosurus.

Pademelon Wallaby, *see* Macropus thetidis.

PERAMELES MACRURA Gould, 1842.

Distribution—Northern Australia, from west to east.

Parasites—

C. Hymenolepis peramelidarum.

C. Linstowia semoni var. acanthocirrosa.

PERAMELES NASUTA Geoffroy, 1804.

Distribution—Eastern Australia.

Parasites—

N. Ascaris sp. Krefft, 1871.

A. Gigantorhynchus semoni.

C. Linstowia semoni.

Perameles obesula, see *Isoodon obesula*.

Peramys americana, see *Didelphis americana*.

Peramys tristriata, see *Didelphis americana*.

PETROGALE PENICILLATA (Gray, 1827).

Distribution—Eastern Australia.

Parasites—

N. Acanthocheilonema australe.

C. Triplotaenia mirabilis.

PHALANGER [*sens. lat.*], unspecified.

Parasites—

C. Bertiella rigida.

PHALANGER URSINUS (Temminck, 1827).

Distribution—Celebes.

Parasites—

C. Bertiella edulis.

C. Bertiella sarasinorum.

Phalangista, see *Phalanger* [*sens. lat.*] which includes *Phalanger*, *Pseudochirus* and *Trichosurus*.

Phalangista vulpina, see *Trichosurus vulpecula*.

PHASCOGALE PENICILLATA (Shaw, 1800).

Synonymy—*Phascologale penicillata*.

Distribution—Australia : widely distributed.

Parasites—

A. Gigantorhynchus sp. Johnston, 1910.

PHASCOLARCTOS CINEREUS (Goldfuss, 1819).
Synonymy—*Phascolarctus cinereus.*
Distribution—Eastern Australia from Queensland to Victoria.
Parasites—
C. Bertiella obesa.
C. Taenia geophiloides.

Phascolarctus cinereus, see *Phascolarctos cinereus.*

Phascologale penicillata, see *Phascogale penicillata.*

PHASCOLOMIS MITCHELLI Owen, 1830.
Distribution—South-eastern Australia, from northern New South Wales, through Victoria, to eastern South Australia.
Parasites—
C. Moniezia bipapillosa.

PHASCOLOMIS sp.
Parasites—
C. Moniezia bipapillosa.

PHASCOLOMIS URSINUS (Shaw, 1800).
Distribution—Flinders and Deal Islands, Bass Straits, Australia.
Parasites—
C. Hepatotaenia diaphana.

Philander, *see* Macropus brunii.

PHILANDER LANIGERA (Desmarest, 1820).
Synonymy—*Didelphys derbiana.*
Didelphys lanigera.
Distribution—South and Central America ; from Paraguay to as far north as Mexico.
Parasites—
N. Cruzia tentaculata.

Philander Opossum, *see* Philander philander.

PHILANDER PHILANDER (Linnaeus, 1758).
Synonymy—*Didelphys philander.*
Distribution—South America : Brazil and the Guianas.
Parasites—
N. Cruzia tentaculata.

N. Echinorhynchus microcephalus.
T. Rhopalias horridus.

Pink-tip-eared Opossum, *see* Didelphis marsupialis aurita.

PSEUDOCHIRUS HERBERTENSIS (Collett, 1884).
Distribution—Central Queensland (Herbert River District).
Parasites—
C. Bertiella aberrata.
C. Bertiella pseudochiri.

PSEUDOCHIRUS LEMUROIDES (Collett, 1884).
Distribution—Herbert River, Central Queensland.
Parasites—
C. Bertiella pellucida.
C. Bertiella undulata.
C. Parabertiella campanulata.

Quenda, *see* Isoodon obesula.

Quica Opossum, *see* Metachirus opossum.

Rat-tailed Opossum, *see* Metachirus nudicaudatus.

Red Kangaroo, *see* Macropus rufus.

Red-necked Wallaby, *see* Macropus ruficollis.

Red-sided Opossum, *see* Didelphis brevicaudata.

SARCOPHILUS HARRISII (Boitard, 1842).
Synonymy—*Dasyurus ursinus.*
Sarcophilus satanicus.
Sarcophilus ursinus.
Distribution—Tasmania.
Parasites—
T. Alaria sp. Cameron, 1931.
C. Anoplotaenia dasyuri.
C. Dasyurotaenia robustae.
N. Nicollina sarcophili.

Sarcophilus satanicus, see *Sarcophilus harrisii.*

Sarcophilus ursinus, see *Sarcophilus harrisii.*

Short-eared Opossum or Phalanger, *see* Trichosurus caninus.

Short-nosed Bandicoot, *see* Isoodon obesula.

Silver-Grey Opossum, *see* Trichosurus vulpecula.

Sombre Wallaby, *see* Macropus browni.

Spectacled Hare-Wallaby, *see* Lagorchestes conspicillatus.

Striped-faced Opossum, *see* Didelphis marsupialis azarae.

Swamp Wallaby, *see* Macropus ualabatus.

Tammar, *see* Macropus eugenii.

Tasmanian Devil, *see* Sarcophilus harrisii.

Tasmanian Wolf, *see* Thylacinus cynocephalus.

Thick-tailed Opossum, *see* Metachirus crassicaudatus.

Thigh-striped Wallaby, *see* Macropus thetidis.

THYLACINUS CYNOCEPHALUS (Harris, 1808).
Distribution—Tasmania.
Parasites—
C. Dithyridium cynocephali.

Tiny Opossum, *see* Marmosa murina.

TRICHOSURUS CANINUS (Ogilby, 1835).
Distribution—Eastern Australia.
Parasites—
N. Filaria sp. Johnston, 1910.

TRICHOSURUS VULPECULA (Kerr, 1792).
Synonymy—*Phalangista vulpina.*
Distribution—Australia, widely distributed, and Tasmania: introduced into New Zealand.
Parasites—
N. Acanthocheilonema australe.
N. Breinlia trichosuri.
N. Filaria dentifera.
N. Protospirura marsupialis.
C. Taenia phalangistae.

Virginian Opossum, *see* Didelphis marsupialis virginiana.

Viverrine Native-Cat, *see* Dasyurus viverrinus.

Wallaby (not specified), includes Dorcopsis, Lagorchestes, Macropus, Onychogalea and Petrogale.

Parasites—

N. Acanthocheilonema australe.

T. Fasciola hepatica.

N. Setaria spelaea.

Wallaroo, *see* Macropus robustus.

Wambenger, *see* Phascogale penicillata.

Water Opossum, *see* Chironectes minimus.

Wied's Opossum, *see* Didelphis marsupialis aurita.

Woodward's Wallaroo, *see* Macropus robustus woodwardi.

Woolly Opossum, *see* Philander lanigera.

Zebra Wolf, *see* Thylacinus cynocephalus.

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Journal of Helminthology, Vol. XI, *No.* 4, *Oct.*, 1933, *pp.* 257-258.

A Note on the Development of *Filaria ozzardi* in *Culicoides furens* Poey.

By J. J. C. Buckley, M.Sc.
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Filaria ozzardi Manson, 1897, or as it is recently but less familiarly known, *Mansonella ozzardi*, was first described in its larval stage, having been found in the blood of Caribs of British Guiana. The adult form, of which the female only was found by Daniels (1899) in the subperitoneal connective tissue of Indians from the same region, is very inadequately described.

The geographical distribution of this parasite is known to include South America and the West Indies. In the West Indies it occurs in St. Vincent, St. Lucia and Dominica (Low, 1902), and it is said also to be present in Trinidad and Barbados. In South America it has been found in Dutch Guiana, northern Argentina, Colombia, and Mexico. In a recent publication McCoy (1933) describes it as a common infection in natives and Indians in Darien Province, Panama. According to Manson and Seligman the microfilaria of *F. ozzardi* has also been seen in New Guinea.

Various attempts have been made to determine the intermediate host of *F. ozzardi* by experimentally feeding insects on the blood of infected persons, but hitherto they have been unsuccessful. Low (1902) in St. Lucia found by direct infection experiments with mosquitos that *Culex fatigans*, *Anopheles albipes* and *Aëdes aegypti* were inefficient as intermediate hosts for this parasite. He also examined numbers of *Pulex irritans* and *Pulex penetrans* from infected persons but found no developmental stages in them. Fülleborn (1908) obtained partial development in *Aëdes aegypti* and *Anopheles maculipennis*. Davis (1928) experimented with bedbugs (*Cimex lectularius*) and Triatomas (*Triatoma infestans*) in Northern Argentina, but found that no larval development took place in

these insects. Experimenting with *Anopheles tarsimaculatus, A. albitarsus* and *Aëdes aegypti*, he was more successful and obtained larvae from the thoracic muscles of these species. In no case, however, was the head invaded by the larvae.

On a recent expedition to the British West Indies the writer examined 69 inhabitants of Calliaqua, in St. Vincent, and found 26, *i.e.*, 37·7% infected with *F. ozzardi*. (Low found 8 out of 30, *i.e.* 26·6%, infected at this spot in 1902.) During June-July 1933, sand-flies of the genus *Culicoides* were collected there, and were fed upon two carriers of *F. ozzardi*, with the result that the development of the worm was followed up in these insects from the early " sausage " stage to an advanced stage in the head. Larvae were found in the head of sand-flies 8 days after the infective blood meal, and emergence of the larvae at the base of the proboscis was easily induced by slight pressure on the head with a needle.

Through the good offices of Sir Guy Marshall the sand-flies used in these experiments have been identified as *Culicoides furens* Poey by Dr. F. W. Edwards.

In a few instances larvae identical with those obtained experimentally, were found as natural infections in sand-flies at Calliaqua, where this insect abounds. These facts, in conjunction with the high infection rate in the endemic area seem to point to *Culicoides* as the vector, and it is not unlikely that it will also be incriminated in other endemic areas.

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Emergence of infective larva of *Filaria ozzardi* from proboscis of an experimentally infected *Culicoides furens* Poey. ($\times 90$).

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New Names in Volume XI.

Corrigenda.

Page 8, line 6 from top, for *Eleophora* read *Elaeophora.*
Page 126, line 5 from bottom, for spingle-shaped read spindle-shaped.

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